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COURTSHIP AND SENSORY PREFERENCES IN INBRED LINES OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The origin of reproductive isolating mechanisms is evidently a problem of great significance for the understanding of the process of species formation. Comparison of full-fledged species permits the study of reproductive isolation which is already established and functioning. One expects however to find within as yet undivided species the genetic materials from which the reproductive barriers between species may eventually be constructed. It is for this reason that several authors have attempted to find the possible beginnings of isolating barriers in the sexual behavior of different strains of *Drosophila melanogaster* (Merrell, 1949 a, b; Spieth, 1952; Knight, Robertson and Waddington, 1956; Bastock, 1956; Koref Santibanez and Waddington, 1958). It appears to us that comparisons of sexual behavior of inbred strains of the same species may be of considerable interest. Inbred strains may differ from each other, and from their outbred ancestors, in several or even in many genes. The present paper reports findings in strains of *Drosophila melanogaster* which have been inbred by brother-sister mating for 414 generations. It will be shown that this inbreeding has allowed establishment of sensory differences which are of consequence in the process of courtship and mating in these flies.

MATERIALS AND METHODS

Three wild type inbred sublines, designated as 3, 13 and 14, were derived from an Oregon line which had been brother-sister mated for 404 generations. At the time of this study each of these three sublines had been brother-sister mated for 10 further generations, i.e., they underwent 414 generations of inbreeding. Two wild type outbred lines, Samarkand and Oregon (the outbred Oregon is, of course, a line separate from the inbred Oregon from which the sublines were isolated), were also used. All the flies were cultured at a constant temperature of 23° C, on the usual cornmeal and sugar medium. Males and females were isolated within a few hours of hatching and used in the observations when four days old. Uniform age may be important to obtain a uniform courtship behavior. Flies of different ages

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may have different sensory thresholds (as found in preliminary trials).

The male choice method (Dobzhansky and Mayr, 1944) was employed. Two females from different lines were placed together with one male of either line in a clean 7×2 cm. vial. One female was marked by a spot of silver paint on her thorax (Knight, Robertson and Waddington, 1956), twelve hours before the observations started. Direct observations were made as described by Spieth (1949). All observations were made at room temperature, 23° C, for a period of 90 minutes. Courtship displays and copulations within this time were recorded. The moment a copulation occurred it was recorded and the vial was laid aside.

Mating Behavior

The nomenclature used here to describe the courting activities is that of Sturtevant (1921) and of Spieth (1947, 1952). Courtship in mature Drosophilids is a highly ritualized behavior (Milani, 1951). After noticing the presence of another fly, the male of *D. melanogaster* performs a series of standard motions. It is unnecessary to describe details of courtship, since other workers have done so for many species (Spieth, 1952). It will suffice to list here the chief events as observed in 1,124 individuals of *Drosophila melanogaster:* The male

1) orients himself toward the passing female;

2) raises his wings as he approaches the female;

3) displays his wings towards whatever part of her body he may be facing. If the female is moving, he will follow her, vibrating his wings. She may stop and accept his courtship. If so, the male

4) displays his wings in front of the female;

5) circles her and moves toward her rear;

6) taps, first with front legs, then with the proboscis on the wing region, and finally on the genitalia region. The male then

7) licks and may mount the female.

8) The mounting occurs if the female does not a) kick, b) flutter her wings, c) runs or flies away, d) leaves by a jerky jump, or e) closes her wings and twists her abdomen toward the inside.

9) If the mounting and copulation occur in spite of the female's refusal, the copulation time will be short, for the female will continue to kick until she frees herself from the unwanted mate. No attempt was made to determine whether these short copulations included ejaculations or not.

The female reacts toward the courting male in the following manner:

1) She may face the male directly, or 2) may move her abdomen as the male displays his wings, or finally 3) she may either stand still to receive the courtship or may leave.

We found the motions of the male which are used to reinforce his courtship behavior to be like those described for *D. subobscura* by Milani (1951).

Excitation Threshold and Sexual Drive

Sexual drive may be regarded as an internal strain released by courtship and mating behavior. However, in Drosophila, the strain does not necessarily disappear after one copulation. In fact, more than one copulation may be necessary (Hoenigsberg and Koref Santibanez, unpublished results). Since sexual drive seems to relieve internal tensions, it may be regarded as a response to internal stimuli (Spieth, 1949).

Results

The time spent in courtship is considered in table 1. Samarkand or Oregon males spent the same amounts of time courting either type of females. Considering the numbers of copulations we found that the Samarkand strain shows a significant tendency toward homogamic matings. On the other hand, in orientations and tappings both strains showed slight but significant preferences toward

TABLE 1. *Courtship behavior of outbred Oregon and Samarkand strains of* Drosophila melanogaster. The courtship time, numbers of copulations, of tappings, and of orientations of 84 outbred individuals, of which 56 were females and 28 were males, are given. The courtship time is expressed as the number of minutes spent in courting the female; the number of copulations is the total copulations of the several pairs; the tappings and orientations are given in total numbers of these events for the same courting pairs.

Courtship time		Own strain		Other strain			
Females	Males	Min.*	%	Min.*	%	χ^2	P
Samarkand, Oregon	Samarkand	6.58	39.0	10.1	61.0	0.72	50 > 30%
Samarkand, Oregon	Oregon	7.70	54.6	6.38	45.4	0.12	70 < 80%
		Number of copulations					
		n	%	n	%	χ^2	P
Samarkand, Oregon	Samarkand	24	72.7	9	27.3	3.40	5 < 10%
Samarkand, Oregon	Oregon	11	34.4	21	65.6	1.56	20 < 30%
		Number of tappings					
		Taps	%	Taps	%	χ^2	P
Samarkand, Oregon	Samarkand	33	66.0	17	34.0	5.12	2 < 5%
Samarkand, Oregon	Oregon	92	63.4	53	36.6	10.50	< 1%
		Number of orientations					
		Orient.	%	Orient.	%	χ^2	P
Samarkand, Oregon	Samarkand	65	60.1	43	39.9	4.48	2 < 5%
Samarkand, Oregon	Oregon	69	50.3	68	49.7	0.007	90 < 95%

* Decimals do not represent seconds but fractions.

females, although the Oregon males oriented indifferently to both types of females (table 1). It may be concluded that in both strains there was a slight preference for homogamic mating.

Table 2 shows that when the males were outbred Oregon there was a very significant preference for copulation with their own females, and a slight tendency toward heterogamic matings when the males were inbred Oregon.

In table 3, in which courtship with each female is taken into consideration, one-half of the outbred males displayed a marked preference for alien females. The other half of the outbred males (Samarkand and Oregon) showed randomness in their approaches to the inbred and to their own type of females. On the contrary, the inbred males displayed a very marked discrimination. They spent significantly more time with their own inbred females.

TABLE 2. *The numbers (n) of copulations observed between outbred and inbred individuals of Oregon origin*

Females	Males	Homogamic n	Heterogamic n	χ^2	P
Outbred, Inbred	Inbred	52	71	2.9	5 < 10%
Outbred, Inbred	Outbred	104	51	17.8	< 1%

TABLE 3. *Courtship preference.* The letter n refers to the total minutes of courtship spent by each kind of male with the female of his choice. A total of 1,254 individuals were observed of which 418 were males and 836 females. The symbol (−) indicates that the males courted more frequently females not of his own line.

Females	Males	Own line		Other line		χ^2	P
		n	%	n	%		
Samarkand, Oregon inbred 13	Samarkand	181.0	46.9	204.2	53.1	1.38	30 > 20%
Samarkand, Oregon inbred 14	Samarkand	62.4	54.0	53.0	46.0	0.76	50 > 30%
Samarkand, Oregon inbred 3	Samarkand	135.1	35.5	234.8	63.5	26.8	(−) < 1%
Oregon, Oregon inbred 13	Oregon	256.6	43.3	335.0	56.7	11.4	(−) < 1%
Oregon, Oregon inbred 14	Oregon	79.2	26.6	217.4	73.4	64.2	(−) < 1%
Oregon, Oregon inbred 3	Oregon	278.5	47.6	305.7	52.4	1.2	30 > 20%
Samarkand, Oregon inbred 13	Oregon inbred 13	136.0	64.6	74.2	35.4	18.2	< 1%
Oregon, Oregon inbred 13	Oregon inbred 13	337.3	81.4	76.9	18.6	164.0	< 1%
Samarkand, Oregon inbred 14	Oregon inbred 14	147.4	87.4	21.2	12.6	94.4	< 1%
Oregon, Oregon inbred 14	Oregon inbred 14	343.1	85.6	57.4	14.4	204.0	< 1%
Samarkand, Oregon inbred 3	Oregon inbred 3	412.1	84.1	77.7	15.9	228.0	< 1%
Oregon, Oregon inbred 3	Oregon inbred 3	400.5	72.9	148.3	27.1	116.0	< 1%

Table 3 shows that while the outbred males used shorter courtships in recognition of their own rather than the inbred female, the inbred males displayed significantly more in courting their own females. The lower mating success the inbred males had (table 2) may accordingly be explained in either of two ways: 1) due to a low athletic ability but not a low sexual drive (Smith, 1956), or 2) due to inbred males experiencing a difficulty in interpreting the acceptance responses of the courted females.

While in orientation (table 4) two groups of outbred Samarkand males showed a slight preference toward their own females, one was random in his choice. The outbred Oregon males which had shown a preference for their own females when the other female was an outbred Samarkand (table 1), now showed a preference towards the inbred Oregon females. However, one group of the outbred Oregon males did not display such preferences in their orientations. In the lower half of table 4 one can see how the inbred males show a remarkable preference for their own inbred females (see χ^2 in table 4).

In our tapping data (table 5), practically the same situation as in the orientations is observed. In this table, the

TABLE 4. *Preferential display expressed as the number (n) of orientations*

Females	Males	Own line		Other line		χ^2	P
		n	%	n	%		
Samarkand, Oregon inbred 13	Samarkand	96	60.3	63	39.7	6.84	< 1%
Samarkand, Oregon inbred 14	Samarkand	40	62.5	24	37.5	4.0	5 > 2%
Samarkand, Oregon inbred 3	Samarkand	108	54.0	92	46.0	1.28	30 > 20%
Oregon, Oregon inbred 13	Oregon	150	51.3	142	48.7	0.22	70 > 50%
Oregon, Oregon inbred 14	Oregon	122	43.4	159	56.6	4.86	(−) < 5%
Oregon, Oregon inbred 3	Oregon	53	36.3	93	63.7	10.9	(−) < 1%
Samarkand, Oregon inbred 13	Oregon inbred 13	139	65.2	74	34.8	19.8	< 1%
Oregon, Oregon inbred 13	Oregon inbred 13	222	68.3	103	31.7	42.5	< 1%
Samarkand, Oregon inbred 14	Oregon inbred 14	103	68.6	47	31.4	20.9	< 1%
Oregon, Oregon inbred 14	Oregon inbred 14	172	69.6	75	30.4	38.1	< 1%
Samarkand, Oregon inbred 3	Oregon inbred 3	148	62.4	89	37.6	14.7	< 1%
Oregon, Oregon inbred 3	Oregon inbred 3	125	56.8	95	43.2	4.08	< 5%

TABLE 5. *Preferential display expressed as the number (n) of tappings by courting males*

Females	Males	Own line n	Own line %	Other line n	Other line %	χ^2	P
Samarkand, Oregon inbred 13	Samarkand	86	58.5	61	41.5	4.20	5 > 2%
Samarkand, Oregon inbred 14	Samarkand	35	63.6	20	36.4	5.20	5 > 2%
Samarkand, Oregon inbred 3	Samarkand	72	60.0	48	40.0	4.80	5 > 2%
Oregon, Oregon inbred 13	Oregon	132	50.5	129	49.5	0.03	90 > 80%
Oregon, Oregon inbred 14	Oregon	91	40.9	131	59.1	7.20	(−) < 1%
Oregon, Oregon inbred 3	Oregon	94	55.9	74	44.1	2.20	20 > 10%
Samarkand, Oregon inbred 13	Oregon inbred 13	133	65.5	70	34.5	19.4	< 1%
Oregon, Oregon inbred 13	Oregon inbred 13	208	70.0	89	30.0	47.6	< 1%
Samarkand, Oregon inbred 14	Oregon inbred 14	85	72.0	33	28.0	22.9	< 1%
Oregon, Oregon inbred 14	Oregon inbred 14	135	69.2	60	30.8	28.8	< 1%
Samarkand, Oregon inbred 3	Oregon inbred 3	76	72.3	29	27.7	21.0	< 1%
Oregon, Oregon inbred 3	Oregon inbred 3	120	65.2	64	34.8	17.0	< 1%

Samarkand males appear to maintain their preferences for their own females. Not so the outbred Oregon males, which show randomness in two cases and a preference to the other female in one case. Again, as in previous tables, the inbred Oregon males show a very significant preference for their own inbred females, regardless of what the other female may be.

It is interesting to note that two grades of courtship have been observed which provoke the acceptance response of the female (Hoenigsberg and Koref Santibanez, 1959). These two degrees of courtship were designated simple and complex according to the number of events included in the ritual. The simple courtship takes place in about one second, and includes licking, mounting and the wing opening response of the female approached. The complex is a prolonged courtship; it includes a series of events (described above) before the acceptance response of the female. Data from both types of courtship indicate that the outbreds made more and preferential use of the simple courtship while the inbreds made more and preferential use of complex courtship in their behavior.

DISCUSSION

The observations described above have disclosed a divergence in courtship and sensory preferences in the discrimination of mates in inbred stocks of *Drosophila melanogaster*. The following differences have been observed: 1) copulatory preference by outbred males (table 2); 2) courtship preference by outbred and inbred males (table 3); 3) significant preference in both distant and proximal stimuli by inbred males for their own females (tables 4 and 5).

As pointed out by Maynard Smith (1958), in species in which males are polygamous, the females are the discriminating partner which decides whether the mating will or will not occur. Our present data can, however, be interpreted in two ways. First, one may suppose that the inbred males, although showing courtship preference, failed to show a concomitant mating preference because of their low "athletic ability," but not due to a low sexual drive. Secondly, the outbred males failed, in many cases, to heed the signaling of rejection by inbred females, and for this reason continued to court them. In the first case (inbred males courting inbred females) the deciding partner in this courtship and copulation acceptance would have to be the male, since the females keep their excitation threshold low. In the second case, however, female discrimination would have to be recognized, since her own threshold depends on the presence of a certain type of male. In this case, there should be a

consistent amount of female rejection as indicated by a lesser number of copulation successes in relation to the duration of courtship.

Taken as a whole, our results point to both sexes being involved in the determination of the courtship success. In all cases where the males were inbred (in a vial with an inbred and an outbred female) the inbred females had longer courtship (table 3), significantly more orientations (table 4) and tappings (table 5), but random copulations (table 2). On the other hand, when the outbred Samarkand or Oregon males (in a vial with their own outbred and an inbred female) courted, there was a significant preference for courting the inbred females in three cases and a random choice in the other three cases, although the outbred males tended to copulate with their own females (see tables 2 and 3). This piece of evidence in itself can be taken to mean that the inbred females whom the outbred males courted rejected them significantly more at the courtship stage after orientations and tappings than before the wing opening of the female. Somewhere between these stages of courtship the females signalled more frequently to the courting outbred males. Since the outbred males cannot be considered low in their "athletic ability" their comparatively few successful copulations with the inbred females must be attributed to the female determining the discrimination of the alien males. Actually, Koopman's experiments (1950), in which natural selection after five generations had altered the discriminating ability of the flies, further point to the determining influence of the female discrimination. Hence, it seems that natural selection in species with polygamous males acts on the threshold barriers of the females to insure isolation.

Regardless of how similar may be the courtship behavior in related taxa, there exists a sensory diversity at an intraspecies level (Spieth, 1952; Hoenigsberg, Koref Santibanez and Sironi, 1959). In this manner inbred females may reinforce the barrier against their original outbred population by increasing the stimulus response threshold towards the alien males. However, this is not a complete barrier, since the vigor of the males may at least in part circumvent their difficulty.

In order for Dobzhansky's (1941) mechanism of natural selection to be effective as the molding factor of sexual isolation, previous geographical or ecological isolation must occur in overlapping populations of races or subspecies. In such hypothetical situations (resulting in a low fitness of the hybrids between evolving groups of one species), natural selection may operate to increase the frequency of homogamic matings (Knight, Robertson and Waddington, 1956). Natural selection may, thus, either reduce the number of encounters between mates, or may cause their matings to be less successful. Furthermore, the factors involved in courtship might be expected to be important in sexual isolation and thereby in speciation, and to be transmissible. Therefore, if there is a sensory divergence controlled at least partially by a complex system of genes in the population, natural selection would have, at the subspecies level, enough material on which to exert its modulating action in erecting a sexual isolation barrier.

Thus even the encounters of mates may be subjected to the effects of natural selection on distant stimuli. In fact, we found in our laboratory conditions that the number of orientations of inbred males is significantly greater towards their own than towards other females.

We have tried to show that there is not only inter-species isolation due to sensory discriminations as demonstrated by Spieth (1952) and others, but also that within the gene pool of a Mendelian population there may exist gene complexes capable of producing sexual or ethological isolation. Our results do not permit us to distinguish between Muller's (1949) and Dobzhansky's (1941) hypotheses as to the possible origins and establishment of species isolating mech-

anisms. Nevertheless, at this point we may say that we do not regard these hypotheses as conflicting with one another, since we consider it improbable that only one mechanism of sexual isolation could have served all species. It seems more likely that the isolation of some species would depend on mutations in "isolating genes" which have selective values "in other respects" (Muller), or owing to natural selection which has acted in a particular situation (Dobzhansky).

Summary

Inbred (414 generations) and outbred Oregon strains of *Drosophila melanogaster* and one outbred strain of the same species (Samarkand) were studied for courtship behavior. The results were: 1) in courtship marked isolation was found; that is, the inbred males exhibited significant preferences for their own types of females; 2) the Oregon outbred males, on the other hand, were indiscriminate in their choice, or preferred the alien females; 3) courtship and copulation tendencies were directed toward their own in Samarkand and Oregon strains of *D. melanogaster;* 4) sensorial discrimination in proximate and distant stimuli was analyzed and was found to be present equally in inbred derivatives from their outbred counterparts. Our results are discussed in connection with the possible evolutionary significance of intra-species courtship preference.

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OSTEOMETRIC VARIATION AND FUNCTION IN BATS

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INTRODUCTION

Since the most extensive and direct evidence of macroevolution, the fossil record of the vertebrates, consists almost entirely of skeletal and dental remains, quantitative studies of these systems are of special interest to the evolutionist. Students of continuous variation of the skeleton have amassed an imposing quantity of data over the past several decades, much of which has been, of necessity, of a purely descriptive nature. Although a general synthesis based on studies of osteometric variation and correlation appears to lie some distance in the future, certain generalizations have emerged which consistently lead to verifiable predictions. Furthermore, there has been an ever increasing tendency to relate numerical values to relationships of genetic and functional significance. The studies of Kurtén (1953, 1957) and Olson and Miller (1958) are recent examples.

One of the most fundamental and readily verified generalizations in osteometrics is the circumscribed nature of the phenotypic variation within a species population in spite of the presumed great potential variability of the population. In terms of the coefficient of variation, values for groups with a definitive adult size (e.g., most mammals and birds) regularly fall within the approximate limits of 2 and 8. The concepts of developmental and genetic homeostasis as developed by Lerner (1954) and empirical data derived from wild and inbred rodent populations (Bader, 1956) suggest that groups with quite different degrees of genetic homogeneity may nonetheless manifest very similar amounts of phenotypic variation. Thus, inferences as to the relative extent of genetic heterogeneity based solely on phenotypic variation must be made with considerable caution. Consistent differences in observed variation can be demonstrated, however, both between different regions of the skeleton and between taxa of higher rank. It is with such disparities that the present study is concerned.

One of the most persistent differences in magnitude of variation between higher categories of vertebrates is that which pertains to birds and mammals. In general birds exhibit a lower variation in osteometric characteristics than that shown by homologous traits of mammals. A greater intensity of centripetal selection on flyers suggests itself as a possible interpretation. With respect to differences related to position in the skeleton, several avian studies have revealed a trend of increased variation particularly in the wing, from the proximal to the distal portion of the appendage. This trend appears to be either lacking, or much less regular, in terrestrial mammals.

In this context an osteometric analysis of bats should be of considerable interest. Will they exhibit magnitudes and trends of variation typical of terrestrial mammals or of birds, or, perhaps, partake of characteristics of both? Will the two appendages, which differ strikingly in morphology and function, reflect this difference in terms of quantitative variation and correlation? The behavior of the first digit of the wing should be of particular significance. Although it is borne on the forelimb, it does not aid in support of the flight membrane (in the species studied) and resembles very closely the digits of the foot in size, shape, and function (fig. 1).

No extensive osteometric study has previously been carried out in the bats.

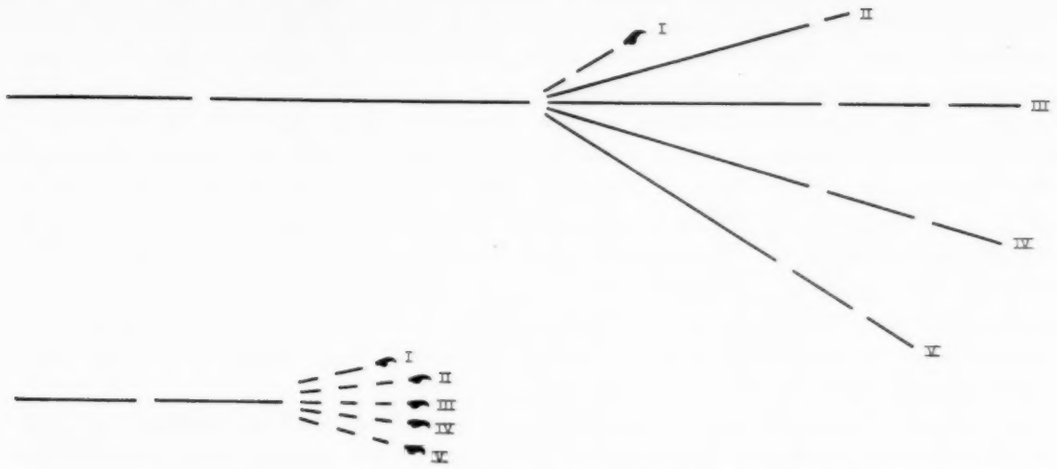

FIG. 1. A schematic representation of the appendicular skeleton of a bat. Digit I of the wing and all digits of the foot bear claws. Relative size of the hindlimb is somewhat exaggerated.

Therefore, two closely related species were chosen in an attempt to more firmly establish osteometric patterns which might be present rather than attempt an analysis of differences between species of widely dissimilar morphology and ecology.

MATERIALS AND METHODS

The bats used in the study were *Myotis lucifugus lucifugus* Le Conte, the little brown bat, and *Myotis sodalis* Miller and Allen, the Indiana bat. Both species were collected from winter hibernating colonies in Coach Cave, Edmonson County, Kentucky, during the winter of 1958–1959. Many thousands of *sodalis* and a few hundred *lucifugus* hibernate in the cave from October through April. They leave the caves in the spring and move to other areas to spend the warm months of the year but the exact pattern of this migration is very poorly known. The evidence at hand (Griffin, 1940) indicates that in the Northeast *lucifugus* may move as much as 150–200 miles between seasons.

The range of *lucifugus* includes most of the forested areas of North America north of Mexico (Miller and Allen, 1928). The range of *sodalis* is restricted to the cave areas of eastern and central United States, from New England to Missouri to Florida (Miller and Allen, 1928; Jennings and Layne, 1957). Coach Cave is thus well within the known range of both species.

Details of the breeding habits are not completely understood. It has been shown by Guthrie (1933) that most females that enter the caves in the fall have been previously inseminated. The sperm remains in the uterus until spring when ovulation occurs (Wimsatt, 1942). Observations of occasional mating in caves by *lucifugus* have been made (Wimsatt, 1945; and our own observations). However, *sodalis* has never been observed mating in the caves during three winters of field study by the junior author in Illinois, Indiana, and Kentucky. Thus, the wintering population of a cave is not necessarily a breeding population.

That the two species resemble each other strikingly is evidenced by the fact that *sodalis* was not recognized until 1928. One may distinguish *sodalis* by the presence of a keeled calcar, smaller foot with very short hairs, and a darker brown color. The skull of *sodalis* is very similar to that of *lucifugus,* but tends to have a narrower interorbital constriction. Although the two forms are almost identical in overall size, some of the more distal elements of both limbs were significantly longer in *lucifugus*. In fact, there was no overlap in the observed ranges in a few of the distal measurements of the hindlimb.

A total of 26 measurements were made on the appendages including all the elements of digits I, II, III and V of the

wing and digits I, III and V of the foot. The unmeasured digits were virtually identical to at least two others which were utilized. The measurements need not be described in detail since all were of essentially the same nature, i.e., maximum length at or near the median plane and parallel to the long axis. In addition two measures were made on the skull, one of maximum length and the other of braincase width at the auditory bullae.

The skeletal parts of the wing and leg were prepared for measuring by skinning and then boiling the intact appendages for about 4 minutes. This was followed by disarticulation of the parts, soaking in 2% KOH for 12 hours, and then storage in water to prevent drying of the finer elements.

The measurements were made with dial calipers, inside micrometer calipers or an ocular micrometer in a dissecting microscope as was appropriate. Measurements over 12 mm were taken to the nearest .1 mm, those less to the nearest .01 mm.

The sample size, which included equal numbers of mature individuals of the two sexes, was 48 for *sodalis* and 44 for *lucifugus*. In each species the sample was analyzed for each sex separately as well as for the total without regard to sex. Since no significant differences between the sexes with respect to either mean size or variation were noted in either species, only statistics derived from the total sample are considered in the following analysis. The statistics were obtained with the aid of the electronic digital computer of the University of Illinois.

Analysis

Variation. The means ($\bar{x}$) and coefficients of variation

$$\left(V = \frac{100 \times \text{standard deviation}}{\text{mean}}\right)$$

for the two species are given in table 1. The values of V for the several measurements fall within the range typical for homologous traits of birds and mammals with the striking exception of that for phalanx 1, II of the wing. The variation in this small terminal element is over twice as great as that of the next most variable element and nearly five times the mean coefficient of the other measures. The median V of all measurements of the two species is 3.95. *M. lucifugus* has a greater V in all but one of the measures but in only one (skull length) is the difference significant at the .01 level of confidence.

Variation in the wing (mean V = 4.47) is somewhat greater than in the hindlimb (mean V = 4.05), even when the highly variable phalanx 1, II (wing) is excluded. Practically all of this difference is due to the more stable nature of the terminal phalanges of the foot as contrasted to the comparable parts of the wing. Variation in total length of a digit was obtained for *sodalis* by adding together the lengths of the constituent parts. These are shown as total I, wing, etc. in table 1. Variation in the sum can be seen to be lower than that in the parts and is slightly less in the leg than in the wing. The lowest variation in both species is exhibited by the two skull measures.

The pattern of variation with respect to the proximal-distal trend in the limbs was virtually identical in the two species. For this reason only the mean of the coefficients for the two species is plotted against position in the appendage. The pattern for the elements of the wing is shown in figure 2. Digits II, III and V which function in support of the flight membrane exhibit a common pattern, i.e., a continuous increase in variation along the proximal-distal axis. The increase is most abrupt in digit II. In these digits, then, the terminal phalanx is invariably the least stable. In digit I an increase is followed by an abrupt drop at the level of the terminal ungual phalanx.

A considerably different pattern is exhibited by the digits of the hindlimb (fig. 3). In digits III and V a very slight increase in variation through the proximal phalanx is followed by a sharp

TABLE 1. *The means ($\bar{x}$) and coefficients of variation (V) of the skeletal measurements of* Myotis sodalis *and* M. lucifugus. Roman numerals refer to digits, arabic figures to phalanges.

	M. sodalis		*M. lucifugus*	
	$\bar{x}$	V	$\bar{x}$	V
Humerus	22.69 ± .10	3.04 ± .31	22.39 ± .09	2.80 ± .30
Radius	36.58 ± .15	2.79 ± .29	35.19 ± .16	3.24 ± .35
Metacarpal I	2.18 ± .01	4.02 ± .41	2.19 ± .02	5.22 ± .56
Phalanx 1, I	3.27 ± .02	4.69 ± .48	3.57 ± .03	5.81 ± .63
Phalanx 2, I	1.69 ± .01	3.28 ± .34	1.83 ± .01	4.80 ± .51
Metacarpal II	32.10 ± .15	3.42 ± .34	30.99 ± .17	4.03 ± .44
Phalanx 1, II	2.32 ± .06	17.51 ± 1.81	2.31 ± .07	20.28 ± 2.16
Metacarpal III	34.29 ± .14	2.95 ± .30	32.92 ± .16	3.60 ± .39
Phalanx 1, III	10.82 ± .06	3.95 ± .40	11.04 ± .09	5.66 ± .60
Phalanx 2, III	9.32 ± .07	5.68 ± .58	9.66 ± .09	6.92 ± .75
Metacarpal V	31.66 ± .13	2.97 ± .30	30.66 ± .16	3.85 ± .41
Phalanx 1, V	7.85 ± .05	4.16 ± .42	8.40 ± .06	4.66 ± .50
Phalanx 2, V	5.05 ± .05	6.67 ± .68	5.20 ± .07	8.92 ± .95
Femur	12.97 ± .06	3.14 ± .32	13.07 ± .07	3.68 ± .41
Tibia	14.26 ± .07	3.44 ± .35	14.69 ± .08	3.74 ± .40
Metatarsal I	1.94 ± .01	4.35 ± .44	2.13 ± .01	4.49 ± .48
Phalanx 1, I	2.52 ± .01	3.94 ± .40	2.89 ± .02	5.13 ± .55
Phalanx 2, I	1.61 ± .01	3.59 ± .37	1.78 ± .01	3.79 ± .41
Metatarsal III	2.16 ± .01	3.66 ± .37	2.42 ± .01	4.50 ± .49
Phalanx 1, III	1.63 ± .01	3.49 ± .36	1.93 ± .01	4.75 ± .51
Phalanx 2, III	1.41 ± .01	5.01 ± .51	1.63 ± .01	6.31 ± .69
Phalanx 3, III	1.68 ± .01	3.40 ± .35	1.81 ± .01	3.65 ± .41
Metatarsal V	2.09 ± .01	3.43 ± .35	2.41 ± .01	4.28 ± .46
Phalanx 1, V	1.69 ± .01	3.48 ± .36	2.01 ± .01	4.37 ± .47
Phalanx 2, V	1.47 ± .01	4.44 ± .46	1.69 ± .01	5.30 ± .57
Phalanx 3, V	1.63 ± .01	2.96 ± .31	1.79 ± .01	4.09 ± .45
Skull length	14.34 ± .03	1.68 ± .17	14.31 ± .05	2.60 ± .28
Skull width	7.39 ± .02	1.96 ± .20	7.72 ± .02	2.05 ± .22
Total I, wing	7.15 ± .03	3.20 ± .33		
Total II, wing	34.46 ± .17	3.45 ± .36		
Total III, wing	54.44 ± .23	2.94 ± .30		
Total V, wing	44.57 ± .18	2.76 ± .28		
Total I, foot	6.06 ± .02	2.83 ± .29		
Total III, foot	6.88 ± .02	2.50 ± .26		
Total V, foot	6.88 ± .02	2.27 ± .23		

increase at phalanx two and by an abrupt decrease at the distal ungual phalanx. The pattern in the first digit is similar but of a modified nature since only two phalanges are present. The trends in the first digit of the wing and the first digit of the foot are similar though not identical, the increase in V through the second phalanx being greater in the former.

Paralleling the increase in V in the more distal parts of the wing is a steady reduction in mean size of the element. In the foot both the progressive increase in V and decrease in the mean are lacking. This suggests the possibility of an inverse causal relationship between the mean and V. The principal rationale for the use of the coefficient of variation as a measure of dispersion is, of course, its quality of greatly reducing, if not eliminating, the highly significant correlation between size and absolute variation. There is, however, no *a priori* reason for assuming perfect direct porportionality between the two. An inverse association between the mean and V has occasionally been noted in other studies (e.g., Alpatov

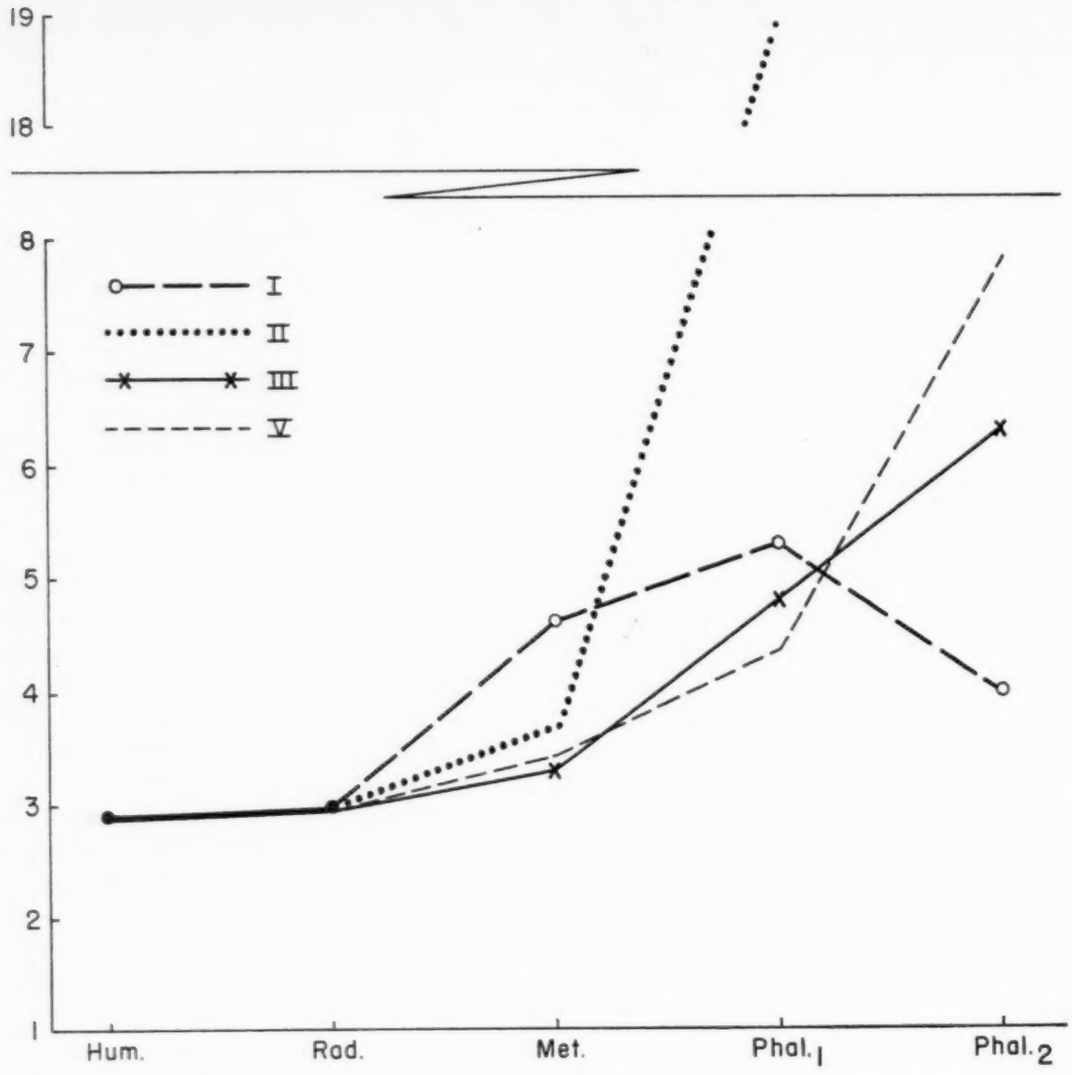

FIG. 2. The coefficients of variation of the elements of the wing arranged according to position in the limb. (See text for discussion.)

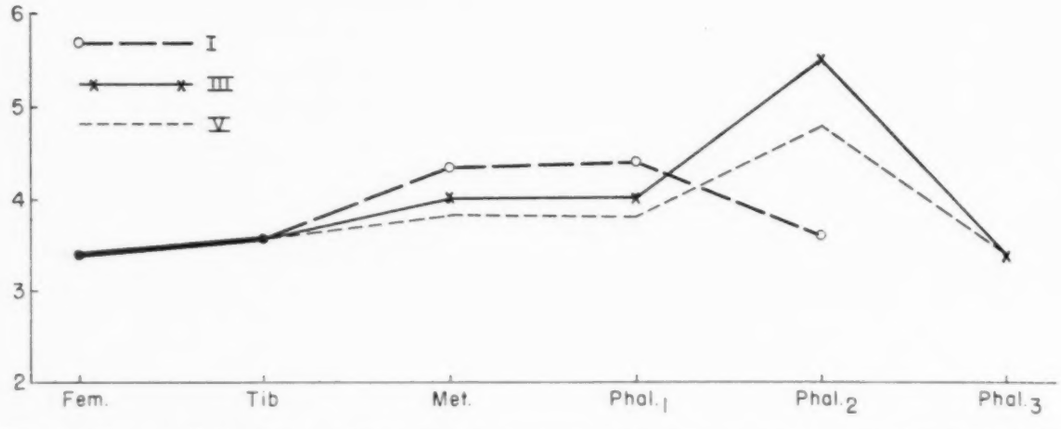

FIG. 3. The coefficients of variation of the elements of the leg arranged according to position in the limb. (See text for discussion.)

TABLE 2. *The coefficients of correlation (r) between the elements of the wing (Digit I excluded) in* Myotis sodalis. Only those values are shown which are compatible with the hypothesis that the population coefficient is $\leq .75$ at the .05 confidence level. Values marked with an * are correlations between elements which occupy the same relative position on different digits.

	Phal. 1, II	Met., III	Phal. 1, III	Phal. 2, III	Met., V	Phal. 1, V	Phal. 2, V
Met. II	—	85*	—	—	77*	—	—
Phal. 1, II		—	—*	—	—	—*	—
Met. III			—	—	89*	—	—
Phal. 1, III				63	—	66*	—
Phal. 2, III					—	—	64*
Met. V						—	—
Phal. 1, V							—

and Boschko-Stepanenko, 1928). Lerner (1958) implies that it may be a factor in the relative magnitude of the variation exhibited by heterotic hybrids and their inbred parents. Data from the overwhelming majority of osteometric studies appears, however, to lend little support to the hypothesis though the possible influence of the mean deserves more attention than it has generally received. The coefficient of correlation between the mean and V for variates of the present study is negative but does not differ significantly from zero at the .05 confidence level in either species.

Correlation. Coefficients of correlation (r) were obtained in *M. sodalis* for all possible pairs of the 28 measurements, a total of 378. In general the coefficients are low as evidenced by the fact that approximately half do not depart significantly from zero at the .05 level of confidence. The range in values is from $+.90$ to $-.25$ with the mean at $+.30$. The degree of correlation between elements in different appendages is, in general, low but of the same order of magnitude as that between elements within either the wing or leg. Intradigital correlations vary tremendously but are not, as a rule, greater than those of a dissimilar nature. Almost all of the correlations between skull and appendage measures are less than .5.

There is, however, one island of order in what is otherwise almost a "sea" of random numbers. That is the correlation between two elements with the same positional relation on different digits of the same appendage, e.g., between metacarpal

TABLE 3. *The coefficients of correlation between the elements of the foot in* Myotis sodalis. Only those values are shown which are compatible with the hypothesis that the population coefficient is $\geq .70$ at the .05 confidence level. Values marked with an * are correlations between elements which occupy the same relative position on different digits.

	Phal. 1, I	Phal. 2, I	Met., III	Phal. 1, III	Phal. 2, III	Phal. 3, III	Met., V	Phal. 1, V	Phal. 2, V	Phal. 3, V
Met. I	—	—	69*	57	—	—	64*	—	—	—
Phal. 1, I		—	—	57*	54*	—	—	54*	59*	—
Phal. 2, I			—	—	—	67*	—	—	—	64*
Met. III				54	—	—	80*	—	—	—
Phal. 1, III					—	—	—	89*	—	—
Phal. 2, III						—	—	—	90*	—
Phal. 3, III							—	—	—	76*
Met. V								—	—	—
Phal. 1, V									—	—
Phal. 2, V										—

TABLE 4. *The coefficients of correlation of digit I (wing) with digit III (wing) and with digit I (foot) in* M. sodalis. Values marked with an * are correlations between elements which occupy the same relative position

	Phal. 1, I	Phal. 2, I	Met., III	Phal. 1, III	Phal. 2, III	Met., I	Phal. 1, I	Phal. 2, I
Met. I	46	2	32*	24	20	73*	48	−7
Phal. 1, I		27	36	38*	41	37	69*	19
Phal. 2, I			18	23	7*	1	18	67*

III and metacarpal V. Most of the higher correlations involve this specific relationship (tables 2 and 3). Phalanx 1, I (foot) exhibits a significant correlation with both the first and second phalanges of digits III and V of the foot with which it shares the same positional relation. Partial correlations with a measure related to overall size, length of the hindlimb, held constant do not materially affect these higher values. The highly variable wing element, phalanx 1, II, has a very low correlation (often negative) with nearly all other parts of both appendages.

The most prominent exception to the general absence of significant interappendicular correlation involves the elements which occupy a proximal position. All six intercorrelations of the humerus, radius, femur and tibia are relatively high ($\geq .56$). However, the greatest values which involve the femur (.83) and the tibia (.73) are with the humerus and radius respectively. Thus the greatest degree of interappendicular correlation also includes elements which occupy the same relative position on the limbs.

Digit I of the wing is, again, of particular interest. It has very few significant correlations with other digits of the wing but shows a high correlation with comparable elements of digit I of the foot. A comparison of the correlation of digit I (wing) with digit III (wing) and with digit I (foot) is shown in table 4.

DISCUSSION

The general magnitude of variation exhibited by the bats (median V = 3.95) appears to fall between typical values for birds and those for mammals though somewhat closer to the former. Median values of V for length measurements in birds often fall in the 2.5 to 3.5 range as indicated by the data of Engels (1938, 1940) for the coot and the thrashers, Fisher (1947) for the condor and Goodge (1951) for the common murre. Although there is a great deal of overlap between the two classes, comparable figures for mammals are commonly in the 5 to 6 range (for a general summary, see Simpson and Roe, 1939). Among the mammals, there are but few well-established instances of significant association between degree of skeletal variation and taxonomic group. Oppossums are highly variable (Bader, 1955) and there is some indication that the variation exhibited by shrews and the smaller rodents is somewhat below the average for the class (Guilday, 1957; Bader, 1956).

As is also true in birds, there appears to be little difference between the two appendages with respect to average magnitude of variation. The hindlimb is not more variable than the wing in either group. The rather high values in the distal part of the bat wing indicate that considerable variation in individual elements is not incompatible with a flying structure. However, variation in total length, which has more aerodynamical and selective significance than variation in the individual parts, is quite low. Such variation and that exhibited by the skull measurements would seem to be close to an irreducible minimum. It is doubtful that experimental procedures such as in-

breeding and artificial selection would be very effective in reducing further such limited phenotypic variation manifested under natural conditions.

The extreme variation exhibited by phalanx 1, II (wing) clearly indicates that the factors which normally operate to control or limit bone development are absent, or at least greatly modified, with respect to this element. The almost complete absence of significant correlation between this phalanx and all other skeletal parts is corroborative evidence. Such variation is common in aspects of the skeleton or dentition which are nonfunctional and presumably under greatly relaxed selective control. It appears to be the only part of the bat skeleton studied which clearly falls into this category.

A band of connective tissue extends distally from the tip of this phalanx along the leading edge of the wing. It may be conjectured that the ossified area is being progressively replaced by the unossified connective tissue. A similar ribbon of connective tissue extends from the end of the other terminal phalanges to the margin of the wing. This tissue makes for greater flexibility of the membrane and reduces the danger of tearing. Thus the same process could be a factor contributing to the high variation exhibited by these terminal phalanges and could, perhaps, be partially responsible for the complete loss of the third phalanx from the digits of the wing.

The proximal-distal trend of increased variation is found only in the digits which are completely associated with the flight membrane. A common, but different, pattern is shared by the digits of the foot. Here the association of variation and position is virtually random. Presumably digits II and IV which are almost identical with those measured would also reflect this pattern. In all digits which bear a claw, the ungual phalanx, although terminal in position, is one of the most conservative parts of the appendage. The pattern in digit I (wing) is most closely similar to digit I (foot) rather than to other wing digits. Clearly, then, the differential patterns of variation are associated with differences in morphology and function.

That the distal increase in variation is common in birds is evident from the data of Engels (1938, 1940), Fisher (1947), Baumel (1953) and others. However a differential pattern between the two appendages has not been clearly demonstrated. Baumel found very similar clines of increased distal variation for the two limbs in the white-necked raven. In other avian studies (e.g., Linsdale, 1928; Olson, 1959) the proximal-distal trend is perhaps more pronounced in the wing but the difference is not great. Among terrestrial mammals a cline has not been clearly shown in either limb although the number of studies which have considered elements distal to the carpus or tarsus is relatively small. The data of Lewenz and Whiteley (1902) for man and that of Olson (1959) for rats fail to reveal a significant association between position and magnitude of variation.

In both limbs of birds and the forelimb of bats, then, relative position in the appendage must be added to the already extensive list of factors known to affect phenotypic variation. In these instances there is a direct association between variation and time of onset of osteogenesis. The more distal elements are under the control of influences, perhaps both genetic and non-genetic, which act later in time and which lead to greater variation. Such a relationship may prove to be a good deal more general than is now recognized if large samples and careful measuring techniques are utilized.

Olson and Miller (1958) have recently treated in considerable detail the relationship between statistical correlation and biological integration from both the empirical and theoretical points of view. Due to the clear-cut nature of the results and the relatively small number of r values, the rather elaborate analysis utilized by them did not seem necessary in the present study. Most of the higher

correlations pertain to the relationship between elements which occupy the same relative position on different digits of the same appendage. The intensity of this correlation is approximately the same within the two appendages and does not appear to vary significantly from the proximal to the distal parts of the digits. The highest interappendicular correlations are between the humerus and femur and between the radius and tibia. It thus appears as if one "field" of influence or integration shared by the two limbs extends distally during development and then cleaves into two at the level of the carpus and tarsus. The more distal areas of common influence may be viewed as segments of concentric rings with a more proximal center. The most significant degree of interappendicular correlation in the distal regions involves the first digits of the wing and the foot. Here correlation and function clearly coincide.

In most studies of correlation and variation within the vertebrate skeleton, one digit, at most, has been measured. The emphasis has been on factors common to parts arranged in tandem rather than those arranged in series at right angles to the longitudinal axis of the appendage. The results of this study would indicate that the intensity of the latter is much the greater.

Summary

Data obtained from an osteometrical analysis of two closely related species of bats, *Myotis sodalis* and *M. lucifugus*, were related to position and function in the skeleton. Median values of the coefficient of variation were more similar to those typical for birds than to those for terrestrial mammals. A pronounced proximal-distal trend of increased variation was demonstrated for those digits of the wing that function in support of the flight membrane. A much more irregular pattern of variation was exhibited by the digits of the foot, the terminal, ungual phalanx being one of the least variable elements. The pattern exhibited by digit I (wing) was most similar to digit I (foot).

Most of the higher coefficients of correlation were between elements which occupy the same position on different digits of the same appendage. Interappendicular correlation was, in general, low except for the more proximal elements. Digit I of the wing showed considerably greater correlation with Digit I of the foot than with any other digit of either appendage.

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A STUDY OF PLANT COMPETITION IN RELATION TO DEVELOPMENT [1]

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Competition as it affects higher plants has been defined by Sakai (1955) as an inter-genotypic interaction. One might amend this definition to state that the interaction is conditioned by the proximity of the individual plants of the population to each other, such that the poorer competitors suffer reduction in the yield of various products, the most notable of which is seed number.

Some experiments on competition in higher plants are available; and though the number of these is not great, they have given convincing evidence for the existence of the phenomenon of competition. However, in all of these experiments that the present author is aware of, definitive data were taken from mature plants. As a consequence of this, some confusion exists as to when during the life histories of the particular species in question, competition was most apt to occur or be most severe. Reference is sometimes made to competition during the seedling stage or at maturity (for example, see Laude and Swanson, 1942).

It would seem correct to assume that if competition does take place it is most likely to occur, or at least have its greatest severity, before the plants are mature, since it is during these stages of development that the greatest demands are being made upon the essential factors in the environment (Clements, Weaver and Hanson, 1929).

The present paper reports the results of experiments designed to study plant development as it affects the ability to compete for the materials necessary for growth. The answers to certain questions were sought:

1. What are the consequences of competition in terms of growth rates and eventual seed yield?
2. What are some of the traits bestowed by the genotype which make for a good or a poor competitor?

The barley varieties Atlas 46 and Vaughn were used as experimental materials since they had a history of being successful and unsuccessful respectively in the selection experiments of Suneson and Wiebe (1942) and Suneson (1949). In addition both are considered well adapted to central California conditions; and both possess distinctive morphological characters such that distinguishing the plants of each in mixtures presented no difficulties.

PROCEDURES

Two types of experiments were performed:

1. Two groups in which the plants were placed 4 inches apart in "checkerboard" plots.
2. Bulk plantings at the rate of about 75 lbs of seed per acre.

The two methods of planting were used in order to compare the results where each plant was known to be surrounded by plants of the second variety, and where neighboring plants were distributed at random with regards to variety. In the spaced plantings competition was regarded as constant from plant to plant; in the bulks as variable.

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In the first two experiments involving regular spacing of plant, there were two treatments; namely, plants grown in pure stands and plants grown in intervarietal mixtures. These were divided into four sub-treatments: Ap—Atlas grown in pure stands, Am—Atlas grown in mixtures, Vp—Vaughn grown in pure stands, and Vm—Vaughn grown in mixtures with Atlas. Such an arrangement of sub-treatments made a 2×2 factorial experiment. The experiments were then laid out as sets of randomized complete blocks. Each complete experiment contained 10 replications, and each of these contained 20 plots placed at random within the replication. The experiment called for five plots of each variety in pure stands within each replication, and the remaining 10 were planted to the varieties in mixture. The varieties were alternated within the mixed stands, i.e., every other plant was Atlas or Vaughn. Each plot contained a maximum of 16 plants to be harvested for experimental data plus sufficient border rows to guard against inter-plot interactions. These experiments were initiated during the fall of 1956 and repeated during the summer of 1957. Germination in both seasons was about 95%. No plot contained less than 14 plants after removal of the border rows at harvest time. One week after germination the plants were thinned to one plant per hill.

At harvest, one plot of Ap, one plot of Vp and two plots of the varieties in mixture were taken from each replication. The Vaughn and Atlas plants from the mixtures were separated and then lumped according to variety. The varietal total from two plots was then regarded as a plot yield. All plots were taken at random from the replications.

Harvests of plant materials were made to correspond as well as possible to stages of development. Atlas and Vaughn showed parallel development as regards stages during winter, but in summer agreement was not so good. This prob-

TABLE 1. *Means and significance levels of estimates for samples from experiment in four inch spacings; winter of 1956–57*

	Ap	Am	Vp	Vm	Significance levels of estimates: Varieties	Treatments	Var. × Treat.
Feb. 25							
Plant wgt.	26.79	28.49	29.05	30.32	**	*	—
Tiller No.	159.91	158.23	186.40	195.31	**	—	—
March 15							
Plant wgt.	55.14	61.68	65.29	59.61	—	—	**
Tiller No.	122.42	132.41	181.45	164.40	**	—	*
April 1							
Plant wgt.	110.22	115.60	116.67	99.07	—	—	*
Tiller No.	76.50	88.70	126.60	108.70	**	—	**
April 15							
Plant wgt.	148.90	183.60	206.00	147.90	—	—	**
Tiller No.	54.60	77.60	90.10	68.10	—	—	**
June 15							
No. heads	48.41	75.62	81.84	62.80	—	—	**
Plant wgt.	175.76	301.40	301.64	213.30	—	—	**
Seed yield (gms. plot)	44.22	68.40	64.59	42.34	—	—	**

TABLE 2. *Means and significance levels of estimates for samples from experiment in four inch spacings; summer of 1957*

	Ap	Am	Vp	Vm	Significance levels of estimates		
					Varieties	Treatments	Var. × Treat.
August 12							
Plant wgt.	5.02	6.10	5.64	5.88	—	*	—
Tiller No.	54.32	63.01	57.60	60.00	—	*	—
August 20							
Plant wgt.	11.83	14.06	11.74	14.68	—	**	—
Tiller No.	91.90	105.90	77.10	87.70	**	**	—
August 28							
Plant wgt.	27.48	34.15	30.43	26.62	—	—	**
Tiller No.	119.00	141.90	99.31	93.52	**	—	**
Sept. 9							
Plant wgt.	75.92	87.77	52.84	41.32	**	—	**
Tiller No.	98.60	122.90	88.50	78.51	**	—	**
Oct. 15							
No. heads	77.00	106.00	86.15	58.90	**	—	**
Plant wgt.	149.10	216.71	100.81	53.41	**	—	**
Seed yield (gms. plot)	66.16	92.96	31.88	17.38	**	—	**

lem is discussed in more detail later. The dates were somewhat arbitrarily chosen, since the developmental sequence of barley will vary in time from season to season. Actually some of these stages extend over periods of several days and tend to merge imperceptibly. In winter the approximate dates corresponding to stages were as follows:

1. Feb. 25—tiller number at maximum; "full tillering."
2. March 15—"jointing," i.e., internodes were undergoing rapid elongation.
3. April 1—flag leaf or "boot" stage; spike ready to emerge from sheath.
4. April 15—mid-anthesis.
5. June 15—maturity; seed ripe.

All green materials were dried in ovens at 150 degrees Fahrenheit and then weighed as quickly as possible.

Analysis of variance was carried out according to procedures outlined by Snedecor (1955). The following parameters, each associated with one degree of freedom, were estimated:

1. *Mean squares for varieties* were used in the comparison of means of varieties regardless of treatments received.
2. *Mean squares for treatments* provided for comparison of materials grown in mixtures and in pure stands regardless of varieties.
3. *Mean squares for varieties × treatments interaction* served as estimates of the degree of inequality of performance of varieties within the different treatments.

The last component represents a competition effect; and, if significant, indicates the failure of a variety to perform at the level of its pure stand control.

The results of these experiments are presented in tables 1 and 2. All weights are in grams, and all estimates are based upon plot yields. Significance levels in the tables are denoted by the use of two asterisks for the 1% level and one asterisk for the 5% level of significance. The bulks were grown and harvested simultaneously with the spaced plantings. A

20 × 20 foot plot of each variety within a treatment was planted adjacent to the spaced plantings. At harvest 30 plants were taken at random from each entry and treated in the same way as the spaced material. Means and standard errors were calculated for each sample and comparisons made among the various subtreatments in a fashion similar to the spaced material.

Results

The results from the spaced experiments showed that in both winter and summer the early development of Vaughn was similar in the mixed and pure stands. At the jointing stage Vm began to show inability to keep pace with its Vp control. This is brought out below in the table on tiller numbers taken from the experiment performed during the winter of 1956–57:

Mean tiller number per plot

	Full tillering	Jointing stage
Ap	159.91 ± 5.29	122.42 ± 3.01
Am	158.23 ± 4.01	132.41 ± 5.65
Vp	186.40 ± 6.83	181.45 ± 3.32
Vm	195.31 ± 4.36	164.40 ± 8.01

Atlas showed a significant tiller loss regardless of treatment. This is regarded as a varietal characteristic. Vm lost about as high a proportion of its tillers as Am or Ap, while Vp suffered very little reduction in tiller numbers over this interval. This suggests that practically all of the tiller loss of Vm was due to the presence of Atlas. Another character which showed evidence of being affected by competition was average tiller weight. The following data were taken at the stages considered above:

Average tiller weight in grams

	Full tillering	Jointing stage
Ap	0.169 ± 0.003	0.476 ± 0.004
Am	0.181 ± 0.002	0.471 ± 0.002
Vp	0.157 ± 0.002	0.360 ± 0.003
Vm	0.155 ± 0.001	0.365 ± 0.004

Gains in average tiller weight may come about through continuing growth or through the elimination of the smaller tillers, or through combinations of the two. Both of these factors were operating here; the relative importance of the two differed in different sub-treatments. The gain in Vp must have been due almost entirely to growth increments, while in Ap and Am both were operating. In Vm there was a significant loss in mean tiller number in comparison with either the previous stage or the adjacent control; yet there was no gain in average tiller weight when compared to Vp. These results are interpreted to mean that growth of the remaining tillers was impeded due to the presence of Atlas.

During the summer Vaughn exhibited an abnormal growth habit. About 12 days after germination the plants began to joint. This occurred before all of the tillers were initiated. Normally in small cereals, and perhaps other annual grasses as well, tiller initiation ceases after the elongation of the culms begins. Small experiments run under greenhouse conditions gave results which suggested that this was a response to day length. Atlas, on the other hand, stooled out completely before jointing, though its life span was much shortened. Due to the peculiar behavior of Vaughn, there was no correspondence between its winter and summer harvests as regards stages. In the case of Atlas, the August 28 sample was taken during the jointing stage, and on September 2 the plants were at anthesis. A significant interaction was detected shortly after Atlas began to joint.

The pattern of competition as related to growth was the same in the bulks as in the spaced plantings, with certain exceptions which will be cited later. This was true in both seasons.

Curves for weight increments and tiller numbers were plotted against abscissas divided into units of equal length (figs. 1 and 2). In both seasons the plants in all entries grew at about the same rate until both varieties in winter and Atlas in summer began to joint. After this the weight curves for Vaughn began to diverge from their controls. In figure 1

it may be noted that the rate of divergence after April 1 is less pronounced until eventually the rate of change for plant weight is about the same in Vp and Vm. In summer the divergence is continuous until maturity indicating the possibility of more severe competition from Atlas during this season. Atlas in both treatments showed a cumulative divergence in both winter and summer, suggesting that Atlas continues to gain in competition with Vaughn throughout its life span. The curves for tiller numbers show much the same pattern as those for weight, though they slope downward.

The bulk of the evidence suggests that most of the competition occurs, or at least has its onset during the jointing stage. A second experiment which served as a test for this hypothesis was performed during the winter of 1956–57.

Plots of Vaughn in pure stands and plots of Vaughn in mixtures with Atlas were planted in sets of randomized complete blocks. As in the other spaced experiments reported herein, the plants were placed four inches apart. On the dates corresponding to the harvests of the other experiments performed that winter, a designated number of plots of Vm were relieved of their Atlas components. Since this procedure provided the remaining Vaughn plants with twice as much space as was available to the Vaughn plants in the unthinned controls, the latter were thinned by removing alternate plants, i.e., the plants whose positions corresponded to those of the Atlas plants in the mixtures. One set of plots were allowed to mature undisturbed to serve as controls for stages. Only the Vaughn plants were harvested. Each determination below is the mean yield of 10 plots, each containing 12 plants (data were taken on seed yields in grams per plot):

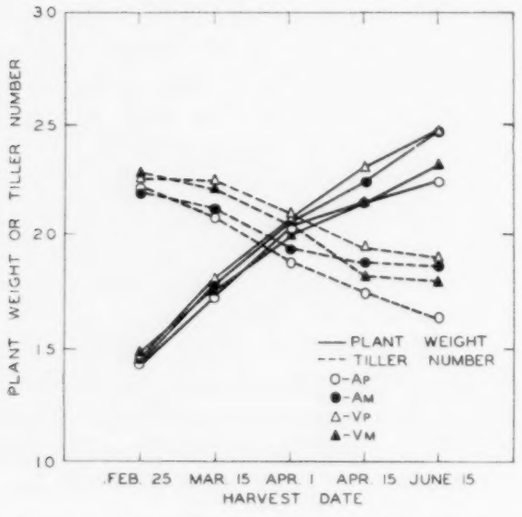

FIG. 1. Changes in plant weight and tiller number in spaced experiment number 1, winter of 1956–57. The ordinates represent $\log_{10}$ mean plot yields.

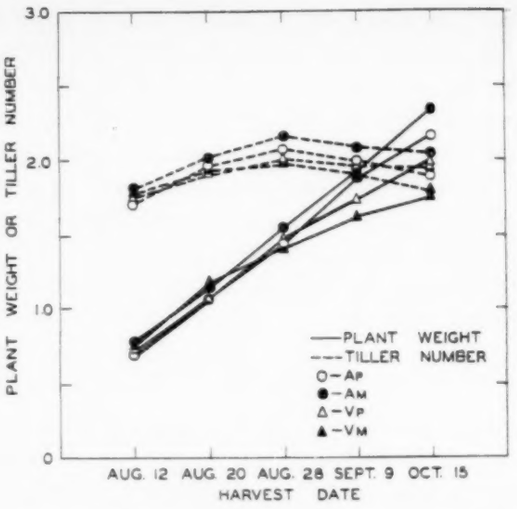

FIG. 2. Changes in plant weight and tiller number in spaced experiment number 1a, summer of 1957. The ordinates represent $\log_{10}$ mean plot yields.

Stage thinned	Pure stands	Mixtures with atlas
Full Tillering	86.92	86.55
Jointing	82.26	58.91
Flag Leaf	71.35	47.87
Unthinned Control	60.89	39.43

L.S.D. (0.5) = 14.54

Analysis of the data revealed that the differences among means of treatments (whether grown in mixed or pure stands) were significant, as were the differences among means of stages. The treatments × stages interaction was not significant.

Graphic presentation of the results (fig. 3) shows that the rate of decrease in yield from one stage to the next is the same after the jointing stage in both

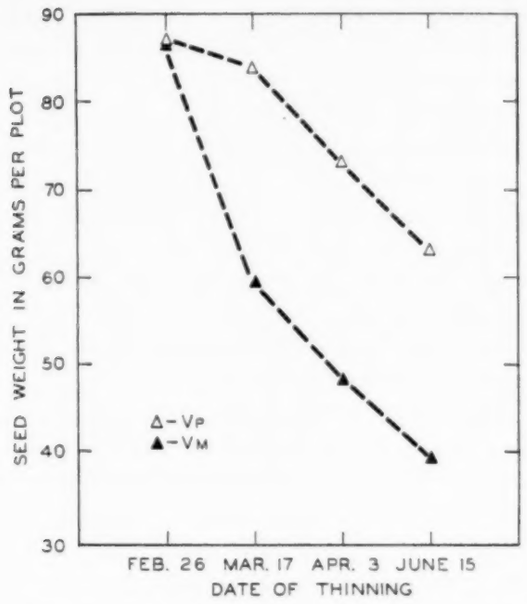

FIG. 3. Rates of decrease in mean plot yields in spaced experiment number 2, winter of 1956–57.

entries. These results are taken as further evidence that the major manifestations occur at a particular stage in development.

STUDY OF ROOT SYSTEMS IN RELATION TO COMPETITION

The abruptness of the onset of competition at a particular stage suggests the occurrence of a specific event in the development of these plants which conditions the inability of Vaughn to realize its potential seed yield in the presence of Atlas. The time to seek such phenomena appeared to be the jointing stage. However, during the summer season Vaughn was already well along into the jointing stage before a significant interaction was detected. This focused the attention upon Atlas as the potential possessor of the critical character differences which condition the onset of competition.

Certain workers in the past have suggested that patterns of root development play an important role in plant competition. Clements, Weaver and Hanson (1929) felt that all parts of the plant were important in conditioning competitive ability, but particularly the roots. Pavlychenko and his associates (1934, 1937) studied entire root systems of crop plants and weeds and came to the conclusion that the rate and extent of root development determines competitive ability in certain annual crop plants. Carter *et al.* (1957) have excellent evidence that the downward growth of roots of cheat (*Bromus secalinus*) is inhibited when grown in mixtures with soft red wheat.

Roots of seedlings and mature plants of Atlas and Vaughn were examined. At 30 days of age (fig. 4) the root system of Vaughn is as well developed as Atlas if not superior to it in number of seminal and branch roots. At this age the shoots of Vaughn are significantly taller and heavier than those of Atlas. At 70 days (fig. 5) differentiation of spikes is complete; and the plants are about to enter the jointing stage. It was at this time that important

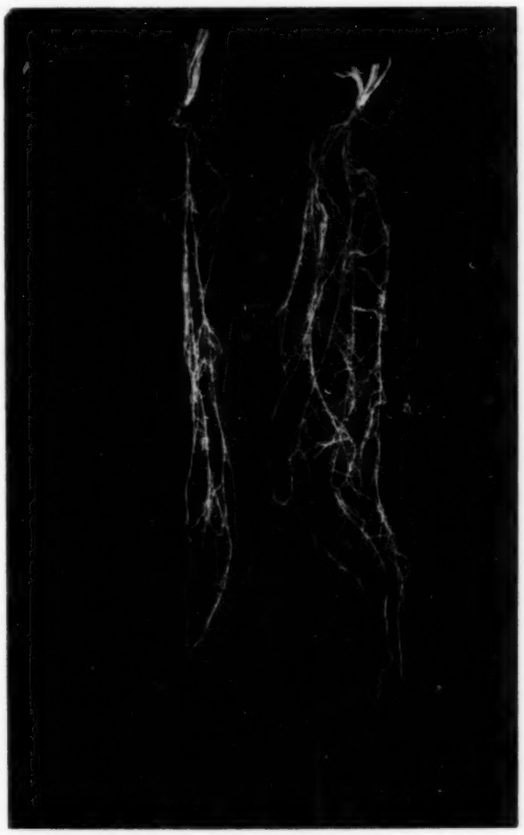

FIG. 4. Root systems of barley at 30 days of age, November 20, 1957; left, Atlas; right, Vaughn. The Atlas plant was the largest of a sample of 12, Vaughn was average for 12. Maximum length of the roots is 14 inches.

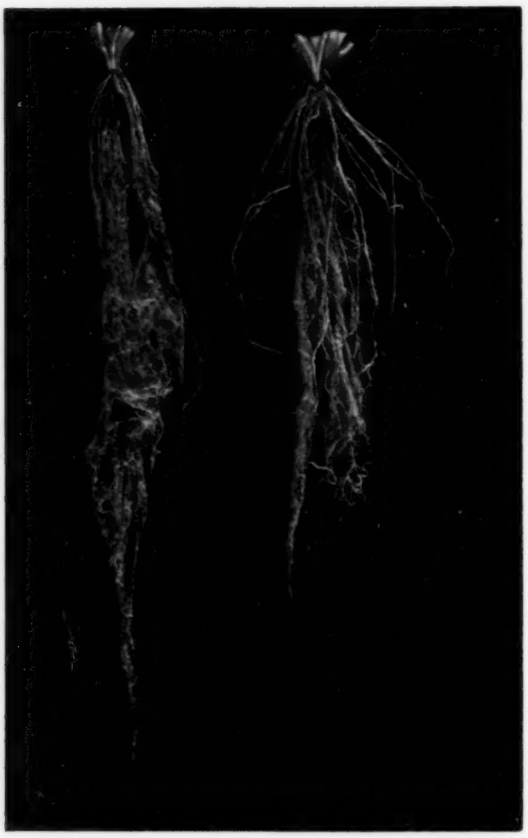

FIG. 5. Root systems of barley at 70 days of age, December 31, 1957; left, Vaughn; right, Atlas. Both plants were of about average size. Maximum length of the roots is 20 inches.

differences in the patterns of root development were noted. Atlas showed numerous large roots growing from the crown. Apparently these roots and their numerous branches eventually assume a large share of the absorptive duties of the plant. Vaughn develops very few of these crown roots; and it appears that most of its root proliferation comes about at relatively deep levels as outgrowths of the seminal roots (fig. 6). Examination of the fine structure of the roots at 70 days revealed that Vaughn had a much more dense crop of rootlets along the seminal roots than Atlas. This has been interpreted to mean that most of the absorbing surfaces of Vaughn are concentrated in "channels" along the seminal roots. Furthermore the root system of Vaughn, while not very dense, is quite extensive. It might depend to a large extent on nutrients, and particularly nitrogen, which move at random into its root channels. Atlas, with its dense, though not particularly extensive, root system would be more efficient in securing the available nutrients within its root zone than Vaughn. It also appears that the entire root system of Vaughn develops more slowly than that of Atlas.

It is thought probable that Atlas, in developing in a relatively short time a dense, shallow root system is able to make heavier demands upon the available nutrients than Vaughn at a time when both are in the act of exploiting heavily a common soil level. Vaughn, here-to-fore impeded only to the degree usually encountered when grown with members of its own genotype, possesses more shoot material than can be nurtured to maturity under the changing conditions. The result is a sudden loss of more tillers and the

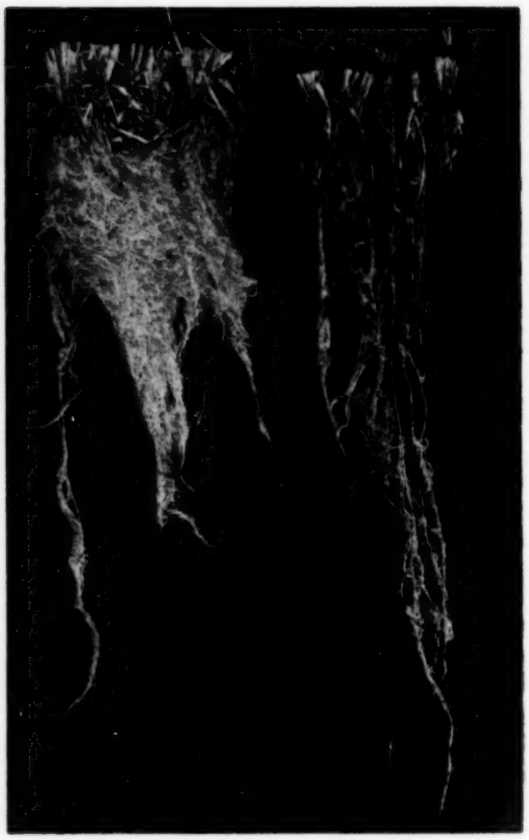

FIG. 6. Root systems of barley at anthesis, September 9, 1957; left, Atlas; right, Vaughn. Each sample contains three plants grown in bulk plots. Maximum length of the roots is 20 inches.

maturing of less vegetative matter in mixture than in pure stands. In winter, at least, Vaughn appears to recover to some extent from the severe initial effects of competition, or at least is able to hold its own after the jointing stage. Possibly it comes to exploit deeper soil levels than Atlas. However, due to the rigidly determinate growth habit of barley coupled with its short life span, there is little or no chance of complete recovery.

Discussion

Harlan and Martini (1938) reported the results of the most extensive experiments on natural selection in higher plants to date. These workers mixed in equal proportions the seeds of 11 strains of barley, and these were then sown at 12 stations in various regions of the United States. Seed for successive generations were harvested in bulk from the experimental plots. Each year samples of seed from each locality were grown out for varietal identification. The duration of the experiment ranged from 4 to 12 years depending upon the location.

At the termination of the experiment it was found that at some stations only a single variety survived in any appreciable number. At all but one station a single variety became dominant.

The following deductions may be made from these results:

1. Competition among the various strains for the essential factors in the environment must have been intense.

2. Competitive ability is related to the environmental conditions under which the population is grown.

The first condition may be inferred since there is evidence that plants of different genotypes often compete when made to grow on the same stratum (Sakai, 1952 and 1955; Sakai *et al.*, 1953 a, b, c and d, 1954, 1955; Frankel, 1939; and the present experiments). The second condition may be related to adaptation to climatic and edaphic conditions as well as to the prevailing epiphytotic situation in a region. It seems safe to say that if a genotype does not convey a high degree of adaptability in relation to these phenomena the strain will not be a good competitor.

There is evidence that adaptation to the factors of the environment mentioned above do not always condition high competitive ability. Harlan and Martini themselves showed that certain locally preferred strains were poor competitors when made to grow with introductions. Sukatschew (1928) produced similar results with dandelion strains in Russia. Montgomery (1912) showed that a high yielding strain of oats was a poor competitor in mixtures with a lower yielding variety. Sakai (1955) states that he could find no evidence that lush growth, high seed yields in pure stands or average height of a strain had any relation to competitive ability. It may be noted that in the present experiments Vaughn in the winter season in pure stands was superior to its Atlas counterpart in all respects. This has been reported before by Suneson (1949).

It would appear then that competitive ability is determined by certain properties which condition the occurrence of an interaction under certain environmental conditions and then only between certain combinations of strains. This is illustrated by the fact that it is possible to have different genotypes which, when grown in mixtures, show no evidence of differential competitive ability. Sakai (1952) found this to be the case with two wheat strains. These properties may be purely physiological as suggested by the findings of Bonner and Galston (1944) and Gray and Bonner (1948). Here it was found that certain perennial plants of arid regions produce in their leaves substances which inhibit the growth of theirs as well as the seedlings of certain other plants. Apparently this mechanism serves to eliminate potential competition. Since competition in this case is a recurring phenomenon most often involving the same species each time, the mechanism is oriented to a specific problem and probably arose in response to it.

Nothing specifically like this appears to be present in the case of Atlas and Vaughn.

Here competition is believed to have its genesis in morphological differences and timing of developmental sequences which eventually lead to physiological consequences. These differences did not arise as a consequence of competition between these particular strains and may not have been primarily determined by intra-specific competition at all, but rather are reflections of adaptation to particular environments. Atlas is known as a Mediterranean barley, having been selected from strains which developed, probably in part by natural and in part by artificial selection, on the dry hillsides of the Mediterranean region. Here the soil is generally thin and rainfall not very dependable. A shallow dense root system would be particularly advantageous in exploiting such an environment. The author knows little of the historical background of Vaughn, but its root system and habit of growth suggests that its ancestors were at least in part developed in the alluvial soils of Egypt and the region known today as Iraq. Vaughn has high potential fitness on soils of this type; and while Atlas will grow perfectly well on these soils, it is not able to give the high seed yields of Vaughn. On the other hand Vaughn is largely a failure on dry hillsides.

It is quite possible that in becoming adapted to thrive in different environments, the ancestors of Atlas and Vaughn did develop considerable ability to compete with other species of plants and particularly weeds. Such competition would involve the problem of the recurring threat as in the case of the desert perennials. It is certainly imaginable that this form of competition could have provided a selective impetus which molded the ancestral strains but in each case in relation to edaphic and climatic factors in the environment.

From the theoretical standpoint at least, intra-specific competition drives the poorer competitors inexorably toward extinction. This may not always be true as Sakai (1955) has pointed out in his demonstration that under competition populations may come to equilibrium with regards to relative frequencies of genotypes. However, his example was based upon sowing rates used in experiments. In nature seeding rates vary from year to year and can be exceedingly dense. Would it not be possible for a particular genotype to be completely without fitness under these conditions? It is worthy of note that between 35 and 40% of the Vm plants from the summer bulks were barren. If the seeding rate had been doubled nearly all may have been without seeds.

It is possible that the ancestors of Atlas and Vaughn were the strains which proved to be the best competitors in their particular environments, and the fact that Atlas proved to be the best competitor when it and Vaughn were planted in mixtures was largely a reflection of its particular line of development in the absence of strains like Vaughn, hence in the nature of a coincidence. Nevertheless these experiments provide suggestive analogies to conditions which could obtain in natural populations. It may be that inter-genotypic competition is indicative of the fact that some genotypes are conveying greater adaptability than others, and as a result of this they are able to secure a larger share of the essential materials than others. The poorer competitors in being eliminated seemingly would be playing a passive role in the shaping of the gene pool. Perhaps here the role of inter-genotypic competition is being cast in a negative vein so far as selection is concerned. This may often be true. Elimination for the unfit is not always a creative process. However, if competitive ability is an indicator of a high level of adaptation, then the elimination of the poorer competitors could in time bring to the level of near or complete fixation those characters which condition high competitive ability and thus greater ability to exploit the environment.

Summary

Experiments were designed to study the effects of plant development on the ability to compete. The barley varieties Atlas

46 and Vaughn were selected for experimental material since they had been shown to be good and poor competitors in selection experiments by previous workers. Pure stands and mixtures of the two varieties were planted in "checkerboard" plots during the winter of 1956–57 and the summer of 1957. The plants were spaced 4 inches apart in the plots. The plots were replicated such that the various treatments could be arranged factorially into sets of randomized complete blocks. In addition bulk plantings were made using both pure stands mixtures in both seasons. Data were taken on dry plant weight and tiller number at intervals during development, and data on seed yield were taken at maturity.

Analysis of these data revealed no measurable effect due to competition until both varieties in winter and Atlas in summer had reached the jointing stage. After the onset of competition Vaughn showed sudden, heavy losses of tillers and a slower growth rate in mixtures than in pure stands. In winter, except for a short period during the jointing stage when competition was most severe, growth rate and tiller elimination proceeded at about the same rate in Vaughn in mixtures as in the control. During the summer the competition effect, once manifest, tended to be cumulative. This was thought to be a reflection of the severe handicap under which Vaughn develops in this season, whatever the treatment otherwise. In both seasons the eventual result of competition was a curtailment of seed production of Vaughn coupled with an increase in the productivity of Atlas in a like degree.

Study of root systems revealed that Atlas begins to proliferate a dense mass of roots from the crown at about the time the internodes begin to lengthen. A parallel root development in Vaughn was not noted. This development suggests that Atlas becomes more efficient in gathering nutrients from a fairly limited area of the soil mass; and, as a result, places Vaughn under severe stress at the time when both are dependent upon the same soil area for nutrients.

On the basis of the foregoing results it is felt that the role of intra-specific competition in natural selection might be, in general, a negative one, but is certainly important in moulding the gene pool in the direction of conditioning greater adaptability.

Acknowledgment

The author wishes to express his gratitude to Professor G. Ledyard Stebbins for his advice and aid in carrying out the research and for critically reading the manuscript and offering helpful suggestions.

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STUDIES ON THE JAPANESE HONEYBEE, *APIS CERANA CERANA* FABRICIUS. VIII. TWO OPPOSING ADAPTATIONS IN THE POST-STINGING BEHAVIOR OF HONEYBEES [1]

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When a honeybee worker uses her sting against a vertebrate enemy, the sting is firmly fixed in the elastic integument of the enemy by the sharp barbs in the tips of lancets. Consequently, to liberate herself, she must tear from her own body the anchored sting, which, together with some associated organs, remains in the wound. This curious autotomy, obviously fatal to the individual which does the stinging, has been regarded by many biologists, including Darwin, as one of the most mysterious functions found in the animal world. On the basis of detailed studies of both the morphology and function of the sting apparatus of the honeybee and related aculeate Hymenoptera, Rietschel (1937) came to the conclusion that this anti-individualistic function is not a mere vestige of a formerly beneficial function, as considered by some previous writers. He believed it to be a real defensive adaptation aimed to secure the maintenance of the colony. To confirm the validity of this conclusion, he presented morphological evidence to be touched upon in part later.

Sting autotomy has hitherto been studied exclusively in the European honeybee, *Apis mellifera* Linne, except for a brief description by v. Buttel-Reepen. His observations are remarkable, because he reported that sting autotomy is avoided in the Indian bee by repeated revolving movements around the fixed sting through which the sting is ultimately detached without tearing of any organs (Maidl, 1933). If such a behavioral adjustment really occurs, and if Rietschel's assumption is valid, it offers a very interesting example not only for the biology of honeybees, but also in respect to the general nature of adaptation. In the course of the writers' comprehensive studies on the Japanese honeybee, *Apis cerana cerana* Fabricius, which is one of the nearest relatives of the Indian bee, *A. c. indica* Fabricius (for our terminology see Maa, 1954; Sakagami and Akahira, 1958; Akahira and Sakagami, 1959), a series of observations was carried out with respect to this problem.

Throughout the studies, all the worker bees of the Japanese species originated from a colony taken in Nagano Prefecture, Central Japan; those of the European bee belonged to the Italian race, *A. mellifera ligustica* Spinola, mixed with various dark-colored strains (as in most colonies kept in Japan). The abbreviations *C* (*A. cerana cerana*) and *M* (*A. mellifera ligustica*) will be used throughout the description of results.

RESULTS OF OBSERVATIONS

1. *Occurrence of sting withdrawal without autotomy*

It was first determined whether sting withdrawal without tearing really occurs or not, and if so, how frequently. For this purpose, the worker bees of the two species were supported at their thorax over a piece of felt 5×5 sq. cm and invited to sting the cloth by causing gentle contact of their abdominal tip with it.

[1] Contribution No. 462 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

[2] The writers are much indebted to Prof. Tohru Uchida, under whose direction the present study was carried out.

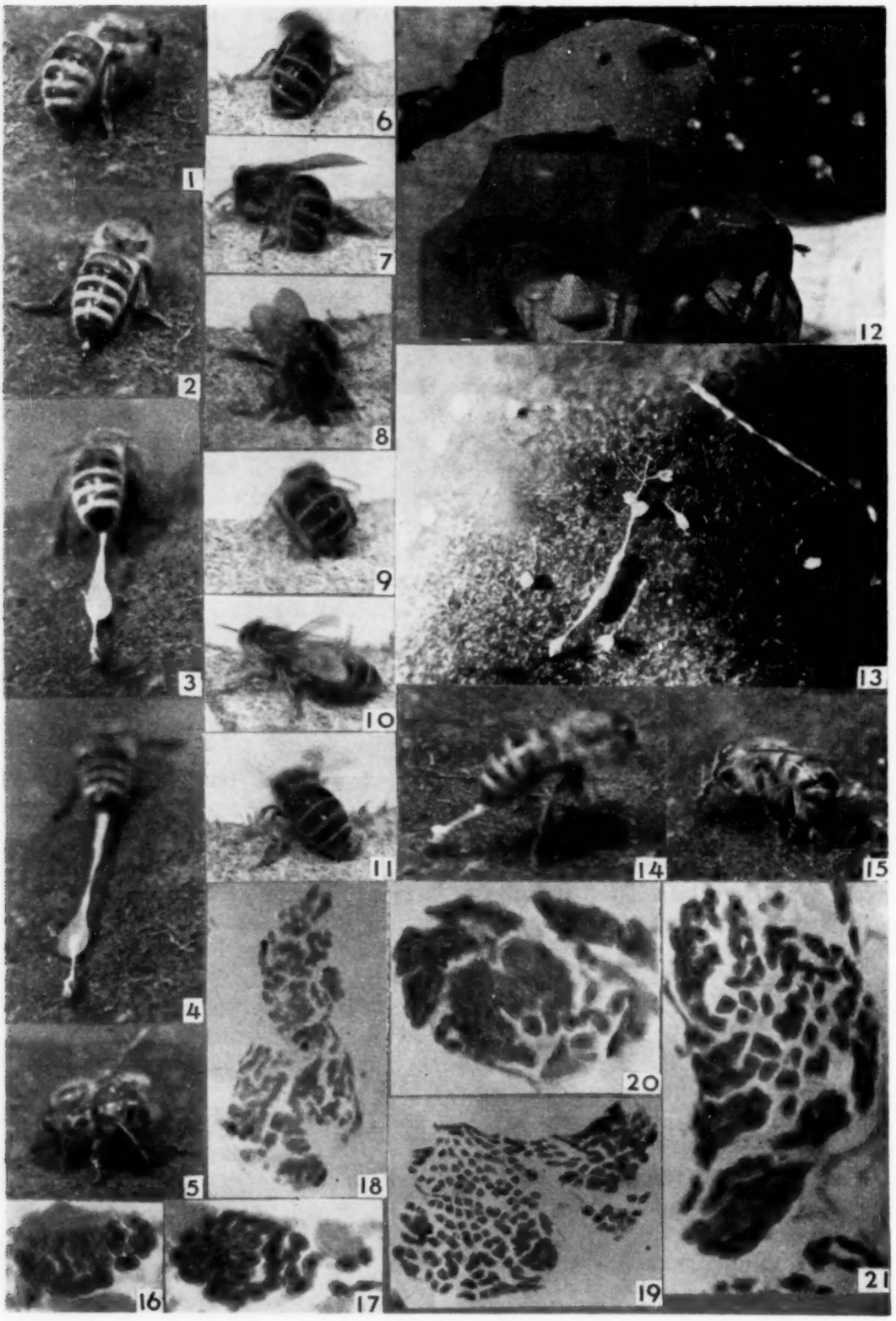

PLATE 1

FIGS. 1–4. Successive steps of sting tearing by *straight run* (*Apis mellifera*).
FIG. 5. *Gnawing* (*A. mellifera*).
FIGS. 6–11. Successive steps of sting withdrawal by means of *revolving* (*A. cerana*).

If they stung the cloth within ten repeated contacts, the response was classified as *stung,* if not, as *not stung.* From the subsequent observation of the sting autotomy, the response type *stung* was further subdivided into *torn* and *withdrawn.* The individuals, classified *not stung,* were released after the trial without repeated testing. In 200 trials with *C* and 100 with *M* workers, the relative frequency of the three response types was as follows: *not stung* (41% in *C* and 5% in *M*), *withdrawn* (47% in *C,* 24% in *M*), *torn* (12% in *C,* 71% in *M*). These results clearly confirm the validity of v. Buttel-Reepen's observation. Furthermore, two additional items of information were obtained as follows: 1) the readiness to sting is far stronger in *M* than in *C*; 2) after stinging autotomy occurs in both species, but the relative frequency is overwhelmingly higher in *M.*

2. Survival of "withdrawn" individuals

The higher rate of *withdrawn C* individuals is mainly due to the revolving movement described in the next section. However, it is still uncertain whether such response really saves the life of the stinging individual, since it may be argued that some organs associated with the sting apparatus are more or less injured in the course of repeated revolving which thus might cause some harmful effects.

For an answer to this question, similar experiments were continued with *C* workers alone. Forty-five *not stung* (N) and *stung but withdrawn* (W) individuals were, together with 13 *torn* workers (T) obtained during the experiments, marked with colored paint and returned to the home hive. Their subsequent survival was as follows: in the evening of the same day: N(45), W(43), T(0); five days after experiment: N(19), W(21); ten days after experiment: N(13) and W(15).

Assuming the average longevity of adult workers as about one month in the active season, the result distinctly shows that the sting withdrawal by means of the revolving movements is significantly effective in saving the lives of workers which had used their weapon.

3. Behavior analysis of post-stinging reaction

After a worker honeybee, either *C* or *M,* inserted her sting into the piece of felt, several behavior patterns occur. They may be classified as follows:

1) *Gnawing:* Gnawing of test substratum by mandibles (Plate 1, fig. 5).

2) *Excited flight:* An excited attempt to fly away accompanied by loud buzzing (Plate 1, fig. 14).

3) *Straight run:* An attempt to escape by straight walking away (Plate 1, figs. 1–4).

4) *Stroking:* Stroking of the fixed abdominal tip with mid- and hind-legs (Plate 1, fig. 15).

5) *Revolving:* Revolving movements around the fixed abdominal tip (Plate 1, figs. 6–11).

Response patterns 1), 2), and 4) appear only occasionally. Among 95 and 118 trials with *M* and *C* respectively, *gnawing* was found 11 times in *C* and

FIG. 12. Accumulation of torn off stings on the hut resulting from the attack of stimulated worker bees (*A. mellifera*).
FIG. 13. Ditto, torn off stings magnified.
FIG. 14. Sting tearing by excited flight (*A. mellifera*).
FIG. 15. *Stroking* (*A. mellifera*).
FIG. 16. Transverse section of muscle no. 14 of *A. cerana,* × 200.
FIG. 17. Ditto, of *A. mellifera.*
FIG. 18. Transverse section of mandibular adductor of *A. mellifera,* × 100.
FIG. 19. Ditto, of *A. cerana.*
FIG. 20. Transverse section of sting muscle no. 19 of *A. cerana,* × 200.
FIG. 21. Ditto, of *A. mellifera.*

TABLE 1. *Interspecific difference between* A. mellifera (M) *and* A. cerana (C) *in the percentage of trials in which* straight run *was observed*

Species	Number of trials			% *straight run* observed			
				in the whole course of trial		at the start of trial	
	torn	*with-drawn*	*total*	*torn*	*with-drawn*	*torn*	*with-drawn*
C	24	94	118	92.0	20.2	83.3(79.2)*	2.1
M	71	24	95	100.0	66.6	64.8(59.2)	20.8

* Per cent of trials in which the sting was torn off by *straight run* which appeared immediately after stinging (the simplest cases).

6 in *M*. *Excited flight* and *stroking* occurred 30 and 9 times in *M* respectively, but never in *C*. *Gnawing* usually appeared immediately after the sting insertion (9 out of 11 times in *C*, 3 of 5 in *M*). The difference between *C* and *M* (not statistically significant) is probably correlated with the specific trait of *C*, which tend frequently to use their mandibles under various situations (see Discussion). *Excited flight*, which is only an exaggerated expression of an escape drive shown in *straight run*, also mainly appears immediately after the sting insertion (21 times out of 30 in *M*). *Stroking* was found only during the repeated attempts to *run straight*, away from the fixed sting, and can be considered either as a trial-and-error type of response or as a displacement activity. The lack of these responses in *C* within the present sample suggests a lower de-

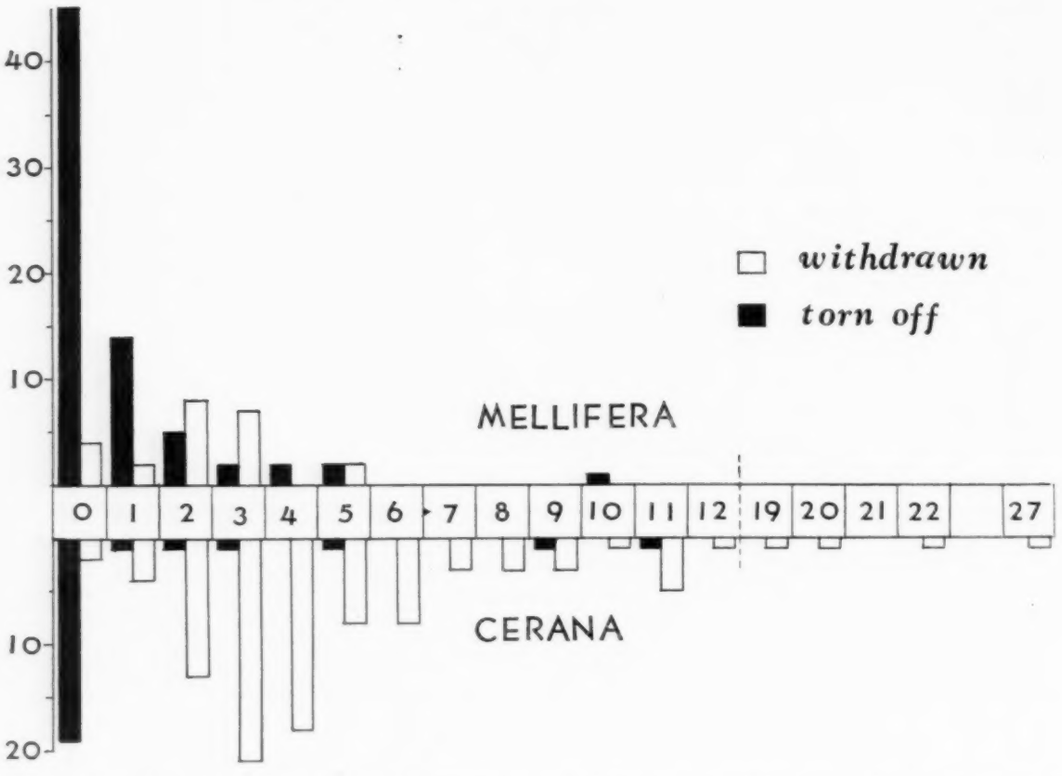

FIG. 1. Specific differences in the frequency of *revolving* and *straight run*. Ordinate: number of trials; abscissa: number of circlings. See text for further explanation.

gree of excitation and may be correlated to the well-developed revolving response in this species.

Straight run is regarded as the simplest expression of an attempt to get away from the fixed point with the maximum efficiency. The relative frequency of the appearance of this pattern is shown in table 1 (see also fig. 1), from which the following conclusions are derived: 1) the absolute number of appearances of this behavior is definitely higher in *M*; 2) this behavior is the principal cause of sting autotomy in both *C* and *M*; 3) it appears very frequently soon after the sting insertion; 4) appearance of *straight run* is more frequent in *M* than in *C* even when the sting is ultimately withdrawn.

Finally, *revolving* is the most interesting response pattern. It consists of repeated clockwise or anti-clockwise circling around the fixed abdominal tip through coordinated leg movements. The revolving is either continuous or interrupted by change of direction and occurrence of other behavior patterns mentioned above. The relative frequency of the appearance of this pattern is presented in table 2, and the distribution of the number of circlings in figure 1. The results may be summarized as follows: 1) the appearance of revolving pattern is overwhelmingly greater in *C*; 2) this behavior is the principal cause of sting withdrawal in both *C* and *M*; 3) *C* tends to revolve sooner than *M*; 4) revolving is repeated most commonly 2–3 times in *M*, 2–4 in *C*, but, *C* may repeat up to 27 times. It may also be noted that the direction of circling does not differ in *C* and *M*.

TABLE 2. *Interspecific difference between* A. mellifera (M) *and* A. cerana (C) *in the percentage of trials in which* revolving *was observed**

Species	% *revolving* observed			
	in the whole course of trial		at the start of trial	
	torn	*withdrawn*	*torn*	*withdrawn*
C	20.8	97.9	12.5	86.2
M	36.6	83.3	18.3	37.5

* The number of trials in which the sting was *torn* or *withdrawn* are given in table 1.

From these analyses of behavior it follows that *straight run* and *revolving* are the principal causes of sting tearing and withdrawal respectively, *M* showing the first and *C* the second much more frequently. This difference is intimately correlated with the marked difference of the ratio of successful sting withdrawal which is more common in *C*. Response patterns of minor importance also differ in the two species.

4. *Specific difference in the resistance of sting to tearing*

Although, as shown above, successful sting withdrawal depends mainly on a behavioral adjustment, it is still premature to conclude that this is the unique cause for inhibition of sting autotomy. The senior writer noted that apparently the *C* sting was more resistant to tearing than the *M* sting, when a straight run was involved. No objective proof of this impression was obtained in the analysis of behavior. Consequently, sting resistance was measured by the method developed by Rietschel (1937) with minor modifications. The two measurements used were resistance to tearing (R) and the strength of sting anchorage (V). Table 3 shows the results we obtained on the basis of 20 workers tested in each species, as compared to Rietschel's results with 10 workers of *A. mellifica* (= *mellifera*). The minor discrepancies between his and our results may be due to differences in experimental conditions, the peculiar ratio of $V > R$, characteristic to the honeybee worker alone, being, however, confirmed.

As may be seen from the table, both R and V were larger in *C* than in *M*, the difference being highly significant ($P \ll 0.01$ in both cases). Consequently, it is clear that both the strength of sting resistance to tearing and of the sting anchorage are higher in *C* than in *M*.

TABLE 3. *Strength of sting resistance to tearing (R) and of anchorage (V)*

	Species	Mean (gm)	Standard deviation	Range	Number
R	*C*	2.3	0.37	1.8–2.8	20
	M	1.5	0.33	0.9–2.0	20
	Rietschel's data	1.7	—	1.1–2.4	10
V	*C*	14.0	2.3	9.7–18.5	20
	M	11.4	1.9	9.0–14.7	20
	Rietschel's data	9.4	—	8.4–10.8	10

The higher value of R in *C* may be considered as another aspect of the weak development of sting autotomy in this species, but it is not always easy to relate the stronger anchorage of *C* appropriately to the mechanism of sting autotomy.

5. *Specific anatomical difference in sting apparatus and mandibular musculature*

The manner of sting autotomy was examined on 45 *torn* workers of each species. The sting apparatus of the honeybees, as in other aculeate Hymenoptera, is connected to the spiracle plates, the modified eighth abdominal tergum on each side of the abdominal end. But, in contrast to other aculeate species, three muscles (nos. 10, 11 and 14 according to Rietschel) connecting the sting apparatus and each spiracle plate are remarkably reduced in strength in the honeybee workers. In all *C* and *M* workers examined, the autotomy always occurred at this preformed breaking point. Closer observation proved that, while muscles nos. 10 and 11 may remain after tearing and their breaking is not always regular, muscle no. 14 is always stripped off with a smooth apical surface from the semi-membranous margin of the spiracle plate, leaving the triangle plate of sting apparatus. The sting apparatus is also connected to other organs located in the abdominal end of the bee and some of them are torn off from the body together with the main part of the sting.

The organs torn off are also relatively constant in both species, being, namely, the poison glands, both acid and alkaline ones, the last abdominal ganglion and the end of the rectum. It is interesting that while the poison glands and the ganglion remain with the sting in a relatively constant manner, the breaking point of the rectum is more variable. Moreover, a definite specific difference between *C* and *M* was observed. While the rectum of all 45 *C* workers tore off near the anus, this occurred only in 25 out of 45 *M* workers. In 16 other cases, the dorsal part of rectum tore off with the sting, and in four workers the whole rectum and parts of the small intestine were torn off. This suggests that *M* is apt to receive more severe injuries from the sting autotomy. But the difference is so subtle that no interpretation can be hazarded concerning its significance.

It is doubtlessly true that sting resistance to tearing mainly depends on the three muscles connecting the spiracle

TABLE 4. *Interspecific difference in strength of muscle no. 14 connecting spiracle plates and sting apparatus**

Species	Width of apical attachment surface: Mean (mm)	Standard deviation	Number of workers examined	Number of muscle fibers: Mean	Standard deviation	Number of workers examined
C	0.312	0.0472	20	40.35	10.90	20
M	0.211	0.0134	20	32.00	5.54	21
t	$8.34 \gg 2.704$ (P = 0.01)			$3.169 > 2.704$ (P = 0.01)		

* Preparations were made by Bouin fixation and Delafield hematoxylin staining.

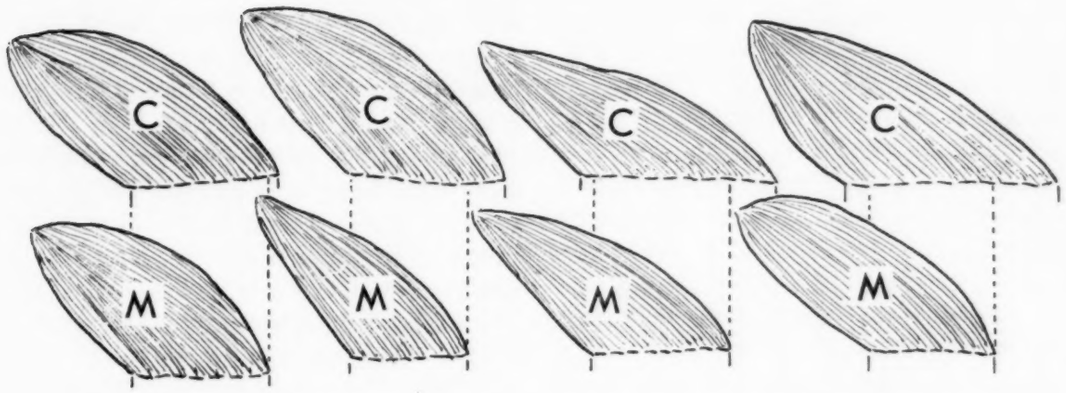

FIG. 2. Comparison of muscle no. 14 in *A. cerana* (*C*) *and A. mellifera* (*M*).

plates and the sting apparatus. However, as mentioned above, these are extraordinarily degenerated in the honeybee workers. Therefore, the strongest and most easily observed muscle among the three, no. 14, was used in strength measurements, noting the width of its apical surface attached to the spiracle plate and the number of muscle fibers. The results are given in table 4 and some examples are illustrated in figure 2 and in Plate 1, figures 16 and 17.

To seek the morphological basis of the stronger sting anchorage in *C* than in *M*, the sting apparatus was examined. In general, in size of numerous parts *M* was larger than *C*. But no difference can be attributed to this fact, since *M* has also a larger body size (Akahira and Sakagami, 1959).

There are no interspecific differences in the number, shape and arrangement of barbs in lancets. On the other hand, a considerable difference was observed in the barbs of the stylet. The difference in appearance of these barbs is so subtle that it is difficult to express it by any numerical values. However, from figure 3, which illustrates the range of variability of barbs in both species in the order of the general conspicuousness (from left to right), it is evident that the barbs are far less developed in *M* in comparison with *C*. In the stylet of *C*, the barbs were always more or less observable and in many workers among 20 individuals examined, each barb was very acute, with a definite posterior incision. In *M*, however, the posterior incision was indistinct even in the few individuals in which relatively sharp barbs are detected. The majority of *M* workers possessed no barbs or only very weak ones. Because of the lack of any detectable difference between the two species in the lancet barbs, it may be argued that the stronger sting anchorage in *C* depends, at least in part, on the developed stylet barbs.

While the sharp barbs in the honeybee lancets are a character familiar to many investigators, the barbs of the stylet have been scarcely mentioned even by the keenest observers such as Rietschel (1937) and Snodgrass (1956), apparently for the reason that they mainly studied *M*. To fill this void, the stylet tips of the following social Aculeata were examined and illustrated in figure 4 (two examples of each species): *Vespa mandarinia* Smith, *Bombus* (*Diversobombus*) *diversus* Smith, *Bombus* (*Bombus*) *spe-*

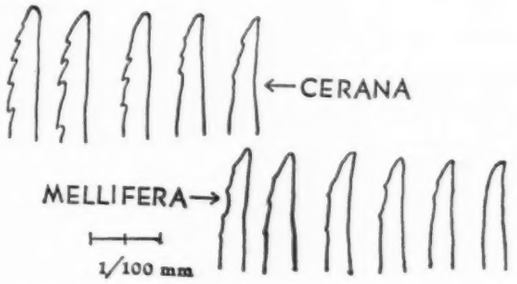

FIG. 3. Comparison of stylet barbs of *A. cerana* and *A. mellifera*.

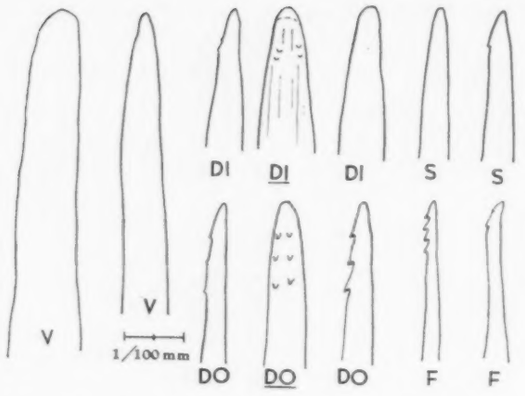

FIG. 4. Stylet tips of some social Aculeata. V: *Vespa mandarinia;* DI: *Bombus diversus;* S: *B. speciosus;* DO: *Apis dorsata;* F: *A. florea.* The views shown are lateral, except when legend is underlined where the view is dorsal.

ciosus Smith, *Apis dorsata* Fabricius and *A. florea* Fabricius. In the formidable sting stylet of the giant hornet, there is nothing which resembles the suggested barbs. In two bumblebee species, relatively remote for each other, the barbs are either absent or very inconspicuous even if any, hence comparable to *M*. On the other hand, two species of Oriental honeybees have relatively well-developed barbs such as in *C*.

Finally, two additional observations were made to demonstrate the anatomical differences corresponding to differences in defensive behavior. It was suggested earlier that the *M*'s readiness to sting is much greater than that of *C*, the latter tending to use its mandibles as a defensive weapon. In order to obtain anatomical data on this difference, muscle no. 19, the most important muscle used in the stinging act, and the mandibular adductor were examined with respect to the number of fibers constituting them. The width of the adductor's apical surface, by which the muscle is attached to the head capsule, was also measured and its ratio to the maximum width of the head was calculated. As shown in table 5 (see also Plate 1, figs. 16–21), there are remarkable specific differences in these values, with a high degree of statistical significance. In particular, *M*'s sting muscle no. 19 possesses nearly twice as many fibers as does that of *C*. Consequently, it may be considered clearly proven that differences in the defensive attitude between the two species are based on corresponding anatomical differences.

DISCUSSION

In social insects of advanced stages, the essential role of self and tribe maintenance under environmental pressure is played by the colony instead of by the individual. In parallel with this alteration of the social (not always biological) unit from the individual to the colony, a number of adjustive mechanisms which are beneficial to the colony but not always to each member of it are found. A general theory to explain the development of such mech-

TABLE 5. *Interspecific differences in strength of sting and mandibular muscles**

Species	Number of fibers in: Sting muscle no. 19 — Mean	S.D.	n	Number of fibers in: Mandibular adductor — Mean	S.D.	n	Ratio of width of apical attachment: Surface of adductor (B) to the maximum width of head (A) — A (mm)	B (mm)	Ratio	n
C	87.75	15.57	12	268.80	51.80	10	3.82	1.34	0.35	15
M	151.78	24.81	14	230.67	35.36	9	4.11	1.11	0.28	15
t	$7.715 \gg 2.819$ (P = 0.01)			$2.696 > 2.567$ (P = 0.02)			$18.046 \gg 2.701$ (P = 0.01)			

* Preparations were made as in muscle no. 14 (footnote to table 4).

anisms was recently presented by Williams and Williams (1957).

Considering the life mode of the honeybee with respect to three major drives, hunger, reproduction and defense, indispensable to self and tribe maintenance, it is obvious that they operate under remarkably different conditions than those of solitary animals. Individual hunger drive cannot be absolutely independent, while the reproductive drive is divided into sexual and parental components which, except for the emergency of queenlessness, are expressed by different castes. But the modification of a drive system attains a dramatic expression in the sting autotomy of the defensive attack. Here the harmful effect on the individual is self-evident by her resultant death.

As already noted, this curious behavior was previously regarded as a vestige of a formerly beneficial function until challenged by Rietschel (1937). It had been well known that the sting anchorage is formed of the sharp barbs in the tips of lancets. As those barbs are observable only in the social Aculeata (Vespidae and Apidae), Rietschel assumed that this equipment adaptively developed in social forms, in order to secure the firm anchorage of sting. The sting anchorage, however, inevitably requires the development of a second adjustive mechanism to liberate the sting from the wound after the injection of poisonous fluid into the enemy. Rietschel found in the social Vespidae such a device in the sting stylet. Here the margin of the stylet end changes to the knife-edge suitable for cutting the fibers with which the lancet barbs are entangled. The stylet of honeybee workers is devoid of such a knife-edge. Moreover, the muscles of workers, which connect the sting apparatus and spiracle plates, are remarkably reduced, offering a preformed breaking point. While these muscles are well developed in other social Aculeata and even in the queens of honeybees, on basis of these observations, Rietschel concluded that sting autotomy is an adaptive mechanism.

It is interesting that the honeybee sting tears off only when used against the elastic integument of mammals and birds. Sting autotomy need not occur when the worker engages an enemy comparable to herself in body size, namely, many insects, because in such cases she has always a chance to win a victory through actual single combat. On the other hand, mammals and birds attack not only the individual workers but also the colony, the social unit of self and tribe maintenance. Here the usefulness of sting autotomy is obvious. In the completely totalitarian system, the loss of hundreds or even thousands of workers may not be seriously damaging, if the colony is well defended. The power of autotomy is well expressed in the difficulty of pulling out the once inserted and torn off sting, together with poisonous glands and ganglion, even with the human fingers which can operate with more coordination than any comparable organs of other vertebrates.

Moreover, although Rietschel did not refer to that fact, it is well known to many beekeepers that an attacking worker is remarkably irritated by the tearing of the sting of her comrade, perhaps by the smell produced thereby. Through this trait, the attack and resulting autotomy are reciprocally accelerated in respect to each other at a geometric rate (Plate 1, figs. 12 and 13). We are therefore inclined to agree with Rietschel that sting autotomy is an aspect adaptive behavior, even though nothing is known about the process through which this curious behavior, being individually harmful and executed by the ***non-reproductive*** females, became established.

If the individually harmful sting autotomy is an adaptive mechanism which is beneficial to the colony, it must logically be also admitted that *the occurrence of any mechanism inhibiting sting autotomy is socially harmful.* In the present paper, it was demonstrated that such an opposite mechanism virtually exists in *Apis cerana cerana* Fabricius. Sting autotomy also appears in this species, but, in most cases,

is avoided by repeated revolving around the fixed abdominal tip. Moreover, this behavior also exists in *A. mellifera ligustica* Spinola, though to a far less conspicuous degree. Therefore, two oppositely operating mechanisms co-exist in both species, but with a reversed frequency of occurrence. This curious phenomenon is discussed below with regard to the significance of revolving and the evolutionary nature of this and related functions.

First of all, an important qualification must be added to the above proposition. The assumption that a mechanism inhibiting sting autotomy is harmful can be justified only in the absence of any other compensatory mechanisms. In two previous papers, the senior writer reported the ethological peculiarities of *A. c. cerana*, as contrasted with *A. mellifera* (Sakagami, 1959a and b). They may be summarized as follows:

1) mild, tolerant and timid nature in relation to human beings, other enemies and to heterocolonial members of the same species. This trait is expressed by the peaceful adoption of other colony members and of *A. mellifera* workers (the reverse interspecific transplantation was very difficult), and by low readiness to sting (corresponding to the weak development of sting muscles shown in the present paper).

2) Pertinacity shown by crawling into clothes and hair of human beings at hive inspection (but never using their sting as frequently as *A. mellifera* under the same situation), and by the persistent attachment to the artificial feeder or to the victim colony when robbing occurred.

3) Frequent use of mandibles observed under diverse situations such as gnawing of human manipulators, hive walls and old combs, and single combat with *A. mellifera*. This trait corresponds to the development of mandibular muscles noted in the present paper.

4) Lack of sedentariness in contrast to *A. mellifera*. Under various conditions, such as food shortage, being robbed, being attacked by enemies, too frequent hive inspections, *A. cerana cerana* abandons the nest frequently in a swarm of the whole colony. This trait is compensated by excellent wax secreting ability.

From these characteristics, it is argued that the sting is not as frequently used by this species as by the European form. Hence, the development of an autotomy-inhibiting mechanism is not always so harmful as suggested above. It is interesting that, of the above characteristics, the second and third, are also well developed in certain stingless bees (Schwarz, 1948) and in numerous ants, that is, in two successful social insects in which the sting apparatus has degenerated.

It is not easy to determine whether or not various mechanisms associated with autotomy-inhibition are adaptively developed. Considering the adaptive evolution of 1) development of lancet barbs (because they are found only in social Aculeata), 2) degeneration of muscles connecting spiracle plates and sting apparatus (because this is found only in honeybee workers) and 3) the resulting development of sting autotomy, while recognizing the possibility of different explanations the following hypotheses are suggested:

1) The revolving has adaptively evolved from the straight run, which is the simplest expression of the escape drive.

2) The less degenerated muscles connecting the spiracle plates and sting apparatus in *A. c. cerana* represented a more primitive condition than that in *A. mellifera*.

3) The reversed relative strength of sting and mandibular musculature is regarded as an adaptation in connection with the development of different defensive attitudes.

4) The less developed stylet barbs in *A. mellifera* are considered as regressive, because this equipment is well developed in three other species of honeybees. But this character should be subjected to further critical study. Undoubtedly, *A. dorsata* and *A. florea* retain many primitive characters. But it is still premature to conclude that both species are primitive in *every* character. The significance of the reduction of stylet barbs is the most enigmatic point concerning the development of two opposing adaptations in post-stinging behavior.

5) The revolving movement must have evolved after the development of the sting anchorage mechanism.

From these considerations, it may be finally concluded that 1) after the considerable development of sting-anchorage, two counteracting mechanisms appeared in social insects—the liberation of the anchored sting by means of the stylet knife-edge in Vespidae and the sting autotomy in the honeybees, and 2) in the course of gradual establishment of this autotomy, another counteracting mechanism, the liberation of the anchored sting by means of revolving movement, appeared. This behavioral adjustment further developed in *A. cerana* together with the less frequent use of sting in defense. In *A. mellifera*, it remained in the rudimentary stage, suppressed and ultimately replaced by the higher development of autotomy, accompanied by an increased reduction of sting-bearing muscles and by the development of sting muscles. Hence, the interspecific difference in the post-stinging behavior suggests that the two species developed their defensive mechanisms in opposite directions.

Summary

1) The interspecific difference in post-stinging behavior was compared between two species of honeybees, *Apis cerana cerana* and *A. mellifera ligustica*.

2) It was found that *A. cerana* is less ready to sting than *A. mellifera*, behavior which corresponds to its weak development of sting muscles. On the other hand, the mandibular muscles are more developed in *A. cerana* in connection with the frequent use of mandibles in this species.

3) Escape by straight run and revolving were found to be the most frequent patterns of post-stinging behavior.

4) Sting autotomy results mainly from a straight run, but is inhibited by revolving in both species. The straight run appeared more frequently in *A. mellifera* than in *A. cerana*, while the revolving is more common in *A. cerana* than in *A. mellifera*.

5) The lack of frequent sting autotomy in *A. cerana* is largely dependent upon revolving. But, in this species, the strength of resistance to tearing and of the sting anchorage are significantly greater than in *A. mellifera*, corresponding to the less degenerated muscles connecting the sting apparatus and spiracle plate and the stronger barbs in sting stylet.

6) The biological and evolutionary significance of the interspecific difference in behavior and anatomy was discussed.

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FOOD PREFERENCES OF LARVAL AND ADULT DROSOPHILA [1]

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INTRODUCTION

Wagner (1944) was the first to show that two species of Drosophila, *D. mulleri* and *D. aldrichi*, differ in their ability to utilize for food certain species of yeast isolated from the breeding sites of these flies. This suggested the possibility that species of Drosophila may be differentiated with respect to their food preferences. The possibility was tested experimentally by da Cunha, Dobzhansky, and Sokoloff (1955) and by Dobzhansky *et al.* (1956) working in California, by Dobzhansky and da Cunha (1955) and da Cunha *et al.* (1957) working in Brazil. In all these studies baits were exposed in the natural habitats of the flies, the baits being inoculated with different yeasts isolated chiefly from the crops of Drosophila collected in the same or different localities. The numbers of wild flies of various species which were attracted to the baits were recorded, and in many cases significant differences between the Drosophila species in the attractivity to them of the different yeast species were observed. Dobzhansky and Pavan (1950) and Pavan (1952) have shown that Drosophila species show also a clear differentiation with respect to the kinds of fermenting fruits and other substances which they select in their natural habitats. Dorsey and Carson (1956) attempted to discover which of the products of fermentation attract Drosophila flies. Of the several varieties of baits used, one, consisting of a molasses-vinegar-water mixture, proved most attractive.

A different approach was utilized by Shehata *et al.* (1955), Carson, Knapp, and Phaff (1956), and Phaff *et al.* (1956). They compared the yeast floras of the known natural breeding sites of Drosophila in California with the composition of the yeasts isolated from the crops of adult Drosophila flies captured in the same localities. They found that the adult flies feed in the main on a different range of the yeast species than their larvae do. No such differentiation was, however, discovered in Brazil (Dobzhansky, personal communication). A still different approach has been that of Dudgeon (1954), who studied the ability of the larvae of the *virilis* group of species of Drosophila to develop in a wide variety of yeasts.

The situation that emerges clearly from the above investigations is, first, that different yeast species are unequal in attractiveness to different species of Drosophila. Secondly, different species of yeast are not equivalent in supporting the growth of Drosophila larvae. The present investigation is primarily an attempt to study the attractivity of different species of yeast to larvae of different Drosophilae. The preference patterns discovered are then compared with the preferences exhibited by the adults of the same species of Drosophila. The only previous work dealing with larval preferences is that of Lindsay (1958), and our results are in good agreement with hers.

MATERIALS AND METHODS

A. Species of Drosophila

Drosophila pseudoobscura: six strains were used in the experiments. Each strain was derived from the offspring of three different females. Three of the strains were derived

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from females collected at Aspen Ranger Station, California, and three strains were derived from females collected at Mather, California. (For descriptions of these collecting sites see Cooper and Dobzhansky 1956.)

Drosophila persimilis: the six strains used were of the same origin as the strains of *Drosophila pseudoobscura,* mentioned above.

Drosophila miranda: six strains were used. Two strains were derived from laboratory strains of uncertain origin. Two strains were derived from laboratory strains collected originally at Big Basin, California, by Th. Dobzhansky. One strain was derived from a single inseminated female collected at Mather and the sixth strain was derived from intercrossing the other five strains.

Drosophila affinis: six strains were used, each derived from the offspring of three females collected on a slime flux on a white oak (*Quercus alba*) at Baldwin, New York.

B. Species of yeast

Ten species of yeast were used in the experiments. These yeasts have been kindly supplied and identified by Professor H. J. Phaff at the University of California at Davis. They are listed in table 1 with their source of isolation. Of these yeasts, No. 197 has been found in both slime fluxes and in the crops of Drosophila (Shehata *et al.,* 1955). *Candida parapsilosis* has been recorded from injured oak trees and from the flowers of the onion and cactus (*op. cit.*). *Kloeckera apiculata* is one of the most commonly occuring yeasts associated with Drosophila having been found frequently in Brazilian species (da Cunha *et al.,* 1957) as well as in California.

C. Larval choice experiments

Three different series of experiments were conducted in which larvae of each species were given a choice of four yeasts. In series II and III one of the yeasts had been included in the previous series of experiments. Thus, series I used yeasts No. 5, 14, 15, 16; series II used yeasts No. 16, 19, 20, 23, and series III used yeasts No. 23, 194, 197, and 201.

The strains of each species were tested separately. Fifty larvae of each strain were used in each of the tests, which were replicated four times. Thus, two hundred larvae were used in testing each strain and twelve hundred larvae were used in testing each species of fly.

Fifty second instar larvae free of gross food particles were placed at the center of a standard (90 mm.) Petri dish containing filter paper

TABLE 1. *The yeasts used in the experiments*

Code No.	Species of yeast	Source of isolation
5	*Saccharomyces dobzhanskii* Shehata *et* Mrak	*Drosophila pseudoobscura*
14	*Hanseniaspora uvarum* (Niehaus)	Juniper berries
15	*S. veronae* Lodder *et* van Rij	*D. pseudoobscura*
16	*S. fragilis* Jorgensen	*D. pseudoobscura*
19	*Candida guilliermondii* (Cast.) Langeron *et* Guerra	*D. pseudoobscura*
20	*C. parapsilosis* (Ashf.) Lang. *et* Talice	*D. pseudoobscura*
23	*C. krusei* (Cast.) Berkh	*Drosophila* sp.
194	*C. pulcherrima* (Lindner) Windisch	Slime flux on *Quercus kelloggii*
197	*C. mycoderma* (Reess) Lodder *et* van Rij	Slime flux on *Quercus kelloggii*
201	*Kloeckera apiculata* (Reess *emend.* Kloecker)	*Drosophila* sp.

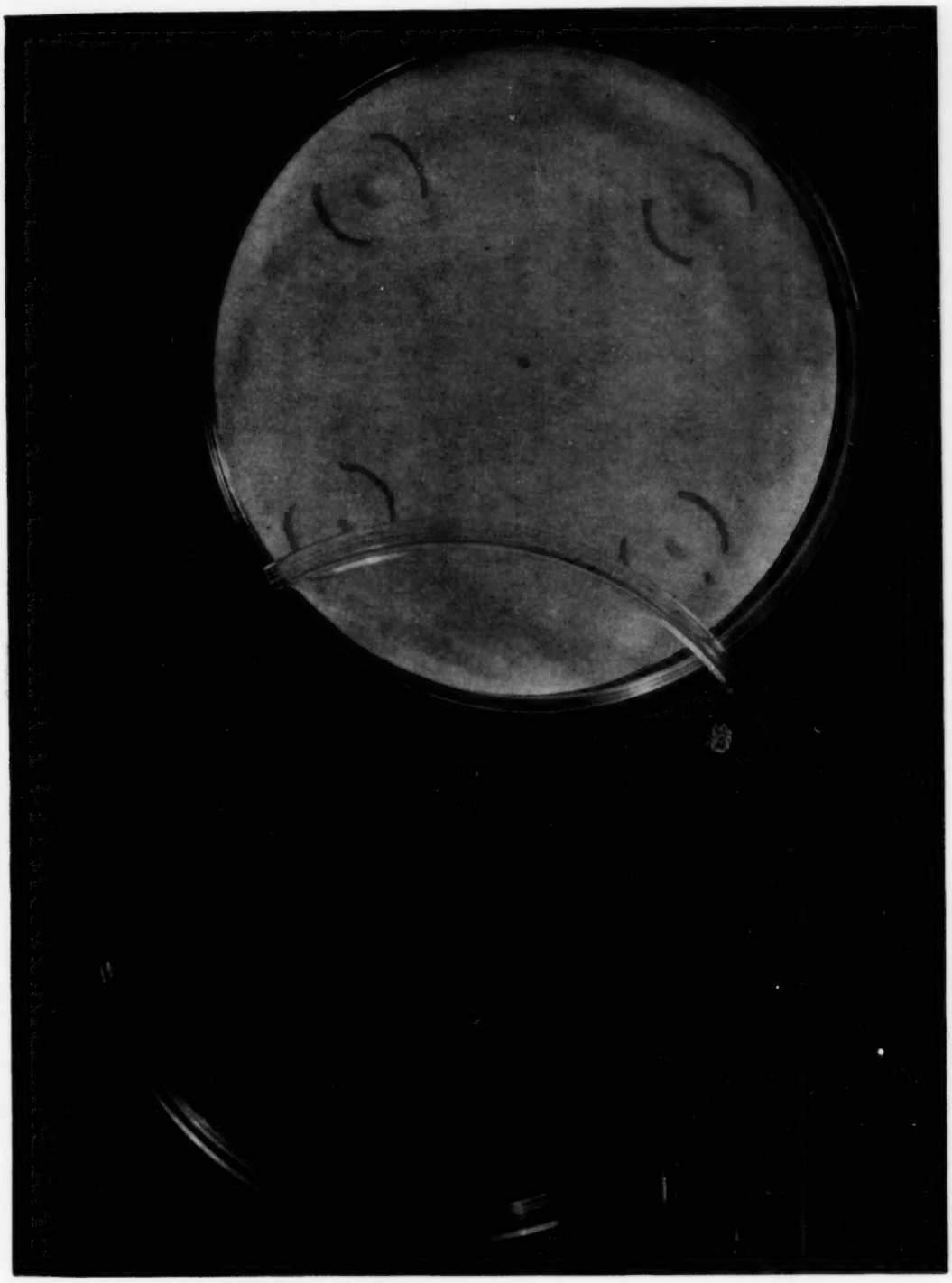

FIG. 1. Petri dish as used in the larval choice experiment showing the counting zones and yeast.

moistened with sterile distilled water. Each Petri dish had previously been inoculated with four yeasts, which were placed in the center of the counting zones indicated by the bracketed areas in figure 1. The amount of yeast in each zone is equivalent to that delivered by a standard bacteriological loop. The Petri dish was then placed in a light tight drawer for one hour. After this time the Petri dish was removed, and the larvae counted in each of the four counting zones. The counting zones were 15 mm in diameter.

The yeasts were cultured on malt agar slants, and the cultures were about two weeks old when used in these tests. The larvae were collected from standard cream of wheat-molasses

food to which 0.5% agar had been added. The food was poured over glass plates 1¾″ × 4½″, seeded with Fleischmann's yeast, and placed in bottles with ovipositing flies from a known strain.

D. The adult choice experiment

The general plan of the experiments was analagous to that used to study the food preferences of the larvae. Three series of experiments were made. Series I used yeasts No. 5, 14, 15, 16; series II used yeasts No. 16, 19, 20, 23; and series III used yeasts No. 23, 194, 197, 201. Each strain of flies was tested once, using 150 adult males and 150 adult females. These flies were derived from cultures in which the flies had aged for one week after emergence. The flies were etherized and placed in a glass vial without food for one to two hours prior to testing. The vials were kept at 17° C. during this time.

Testing of the flies consisted in placing them in cages illustrated in figure 2. This cage is made of a 1½ pint polyethylene refrigerator food container; the container has holes in each side, large enough to take a # 7 rubber stopper. These stoppers were bored to take a test tube 15 × 150 mm., as shown in figure 2. Each test tube contained a two week old slant culture of one of the four yeasts. The top of the cage has a hole covered with fine mesh screen, for ventilation, and two access holes plugged with corks.

Six cages, one for each stock of a species, were placed on the top of a desk in a room lighted by a single ceiling light directly over the cages. Test experiments did not indicate any bias on the part of the flies for any of the four directions in which the test tubes pointed. In each experiment the same yeast always pointed in the same direction. The flies were given one hour to pass from the cage to the test tubes with the yeasts. After one hour the test tubes were removed and plugged, and the flies in each tube were etherized and counted.

E. Larval growth experiments

Preliminary experiments have been made to test the suitability for the growth of the larvae of *Drosophila pseudoobscura* and *Drosophila persimilis* of some of the yeasts used in the experiments described below.

The ability to successfully reach the adult stage has been used as a criterion of effective growth. No attempt to measure the growth rates has been made.

The larvae developed from eggs that were

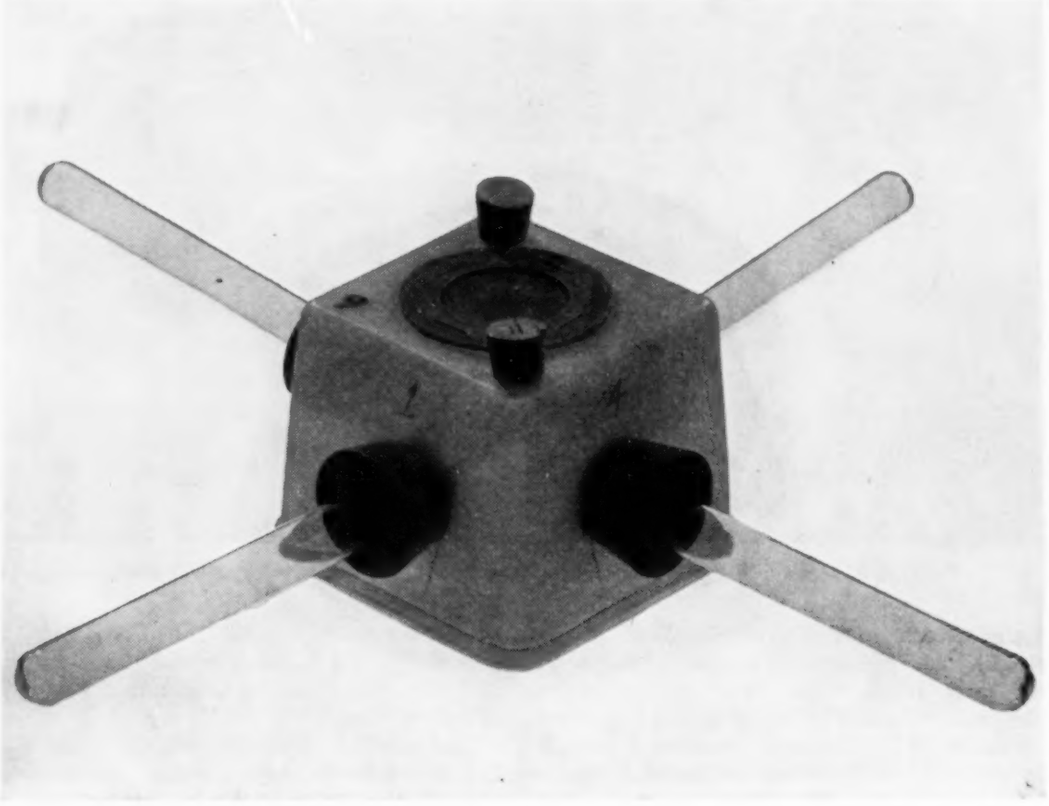

FIG. 2. The cage used in the adult choice experiment.

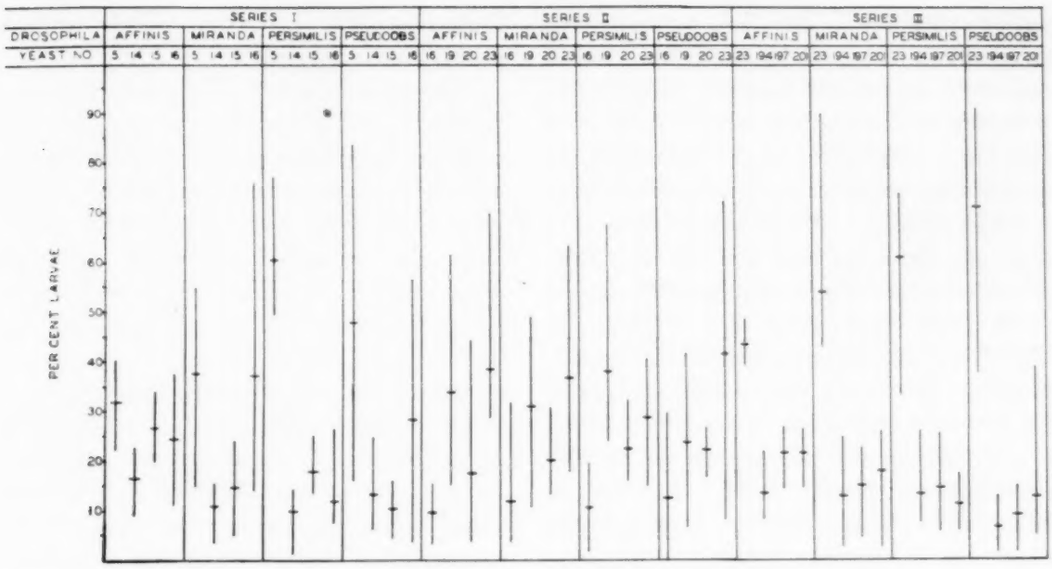

FIG. 3. The results of the larval choice experiments, showing the mean percentage of the larvae of each species of Drosophila choosing a given yeast. Also shown are the ranges of the mean percentages for the six strains of each species of Drosophila used.

washed free of food and microorganisms in a solution used by Begg and Robertson (1950). It consisted of the following: ethyl alcohol (50%) 250 ml, hydrochloric acid 1.25 ml, sodium chloride 6.5 gm, mercuric chloride 0.5 gm, and water to make one liter. The eggs were washed twice in the above solution for periods of 10 minutes, then in sterile distilled water for five minutes, and finally transferred to the culture tubes. Ten eggs were placed in each tube. The eggs were examined after twenty-four hours to check for hatching. If less than 70% of the eggs hatched the tube was discarded.

The culture tubes contained sterile cellulose tissue moistened with 2.0 ml, of a yeast suspension. The yeast was cultured in 75 ml malt extract broth to which yeast nitrogen base (Difco) and glucose were added. The flask containing the culture was maintained at 25° C for 48 hours and was constantly agitated during this time. The broth cultures were then centrifuged. The centrifugate was twice resuspended and washed in 75 ml of sterile distilled water. The washed cells were then suspended in 10 ml of sterile distilled water prior to being placed in the culture tubes. The larvae thus fed on yeast cells and not on any food medium in which the latter may have grown.

RESULTS

A. Larval choice experiments

Two points clearly emerge from these experiments. First, Drosophila larvae when given a choice of several yeasts may show decided preferences. The preference depends upon the repertory of the yeasts offered; the yeast preferred in one experiment may not be the chosen yeast when it is offered together with three different species of yeast. Second, although the pattern of preference for a given yeast is in general similar for the different Drosophila species, clear differences do occur between Drosophila species with respect to the same yeast.

The results of these experiments are summarized in figure 3. In this figure the mean percentages of the larvae going to each yeast are shown. Also shown in this figure are the ranges of the mean percentages for the six strains of each Drosophila species.

To test the preferences for the four yeasts used in each series of experiments, the four yeasts were paired in the six combinations possible (table 2). For each pair of yeasts the mean difference was determined and subjected to a t test with five degrees of freedom. The four yeasts used in each series of experiments yield 6 comparisons. Thus, for all yeasts and all four species of Drosophila there are 72 comparisons, and by chance alone on the

average there will be 3.6 comparisons significant at the 5% level. This should be allowed for in considering the results. Furthermore, the 6 comparisons in a given series are based on only 3 independent observations, so that the comparisons are not independent. This dependence will not affect the expected number of comparisons that are significant by chance, but will increase the probability of two or more of these chance significant results occurring in the same series of experiments.

In the first series for *Drosophila affinis* the only significant difference in preference occurs between yeasts 5 and 14. *Drosophila miranda* shows a significant preference for yeast 5 when compared with yeasts 14 and 15, the difference being significant at the 1% level. *Saccharomyces fragilis* (16) while having a mean preference close to that of *S. dobzhanskii* (5), shows a more variable attractiveness. On the whole, it is not significantly different from 14 and 15. *D. persimilis* exhibits a clear-cut preference for *S. dobzhanskii* over the other three yeasts used in this experiment. The level of significance is better than 0.1%. There is also a significant difference indicated between *Hanseniaspora uvarum* (14) and the preferred *S. veronae* (15).

The response of *D. pseudoobscura* to yeasts 5 and 16 is highly variable. Although both these yeasts seem to be more attractive than either yeast 14 or 15, only yeast 5 proves to be significantly so.

In the second experiment the yeasts used were *S. fragilis* (16), *Candida guilliermondii* (19), *C. parapsilosis* (20), and *C. krusei* (23). The pattern of preference of all four species is very much the same in this experiment. Yeasts 19 and 23 are most favored by all species of Drosophila. Yeast 20 is less often chosen and *S. fragilis* (16) seems to be the least preferred of the four yeasts. *D. affinis* is more attracted to either yeast 19 or yeast 23 than to yeast 16. The difference in attractivity in these two pairs of yeast (19–16 and 19–23) is significant at the 5% level. *D. miranda* seems to prefer yeasts 19 and 23 to yeast 16. The difference, however, is not significant. *D. persimilis* shows significant differences in preferences for all three yeasts than for *S. fragilis*. With *D. pseudoobscura*, however, the variability of the various strains again prevents any significant differences

TABLE 2. *The statistical significance, as measured by the* t *values, of the observed differences in the attractivity of the different species of yeasts to larvae of Drosophila used in the three series of experiments*

	Yeast:	5–14	5–15	5–16	14–15	14–16	15–16
Series I	Drosophila:						
	affinis	4.39	1.31	0.96	2.43	1.33	0.40
	miranda	5.16	4.67	0.03	2.32	2.12	1.74
	persimilis	8.41	8.55	7.71	3.05	0.59	1.46
	pseudoobscura	3.39	3.38	0.99	1.01	1.29	1.82
Series II		16–19	16–20	16–23	19–20	19–23	20–23
	affinis	2.98	1.52	3.92	1.31	0.41	2.03
	miranda	2.55	1.33	2.54	2.33	0.42	1.67
	persimilis	3.36	5.35	3.40	1.87	0.92	1.12
	pseudoobscura	1.76	2.10	2.03	0.24	1.20	1.82
Series III		23–194	23–197	23–201	194–197	194–201	197–201
	affinis	10.00	7.59	9.86	2.91	2.16	0.02
	miranda	4.29	4.14	3.47	0.59	1.54	1.27
	persimilis	5.54	5.23	6.68	0.37	0.87	0.95
	pseudoobscura	7.61	6.59	4.65	1.49	1.17	0.83
		$t_{.05} = 2.57$		$t_{.01} = 4.03$		$t_{.001} = 6.86$	

TABLE 3. *Relative preference shown by the larvae of species of Drosophila for the ten yeasts used in the experiments*

Drosophila:	*affinis*	*miranda*	*persimilis*	*pseudo-obscura*
Yeast:				
5	31.9	37.5	60.7	47.9
14	16.7	10.9	9.9	13.3
15	26.8	14.5	17.5	10.4
16(1)	24.5	37.0	11.8	28.3
19	85.4	98.0	42.2	52.8
20	45.0	64.2	25.2	49.6
23(1)	157.8	116.4	32.1	93.4
194	30.0	27.4	7.0	8.8
197	47.9	32.1	7.6	11.9
201	48.1	38.1	5.9	16.9

from appearing. The preference for yeast 19 appears to be less well marked in *D. pseudoobscura* than in the other species.

The yeasts used in the third experiment were *Candida krusei* (23), *C. pulcherrima* (194), *C. mycoderma* (197), and *Kloeckera apiculata* (201). Here the pattern of preference is very similar for the four species of *Drosophila,* yeast 23 being greatly preferred by all four species. For *D. affinis* the preference for yeast 23 is significant at the 0.1% level when compared with the other yeasts. There is also a possibly significant difference between yeasts 194 and 197. *D. miranda* shows a preference for yeast 23 only slightly less marked than *D. affinis.* The same is true of both *D. persimilis* and *D. pseudoobscura.*

A comparison of the preferences of the larvae for all ten yeasts in the three series of experiments is presented in table 3. The comparison is possible since each series of experiments has one yeast in common with one of the other series. Thus, series I and II both use yeast 16, and series II and III both use yeast 23. The numbers given in table 3 for yeasts 5, 14, 15, and 16 are the mean percentage of larvae of a given species of Drosophila choosing each yeast. The numbers given in table 3 for yeasts 19, 20, and 23 are the mean percentages for these yeasts multiplied by a factor equal to:

$$\frac{\text{mean percentage for yeast 16 (series I)}}{\text{mean percentage for yeast 16 (series II)}}$$

The numbers given yeasts 194, 197, and 201 are the mean percentages for these yeasts multiplied by a factor equal to:

$$\text{the mean percentage for yeast } \frac{16(\text{I})}{16(\text{II})} \times \frac{23(\text{I})}{23(\text{II})}.$$

The significance of this comparison will be discussed in the section on the larval growth experiments.

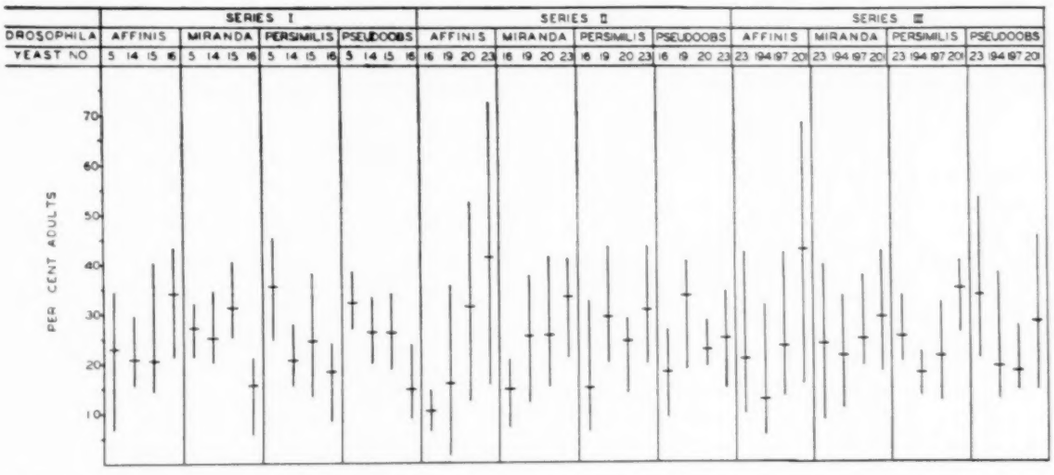

FIG. 4. The results of the adult choice experiments showing the mean percentage of adults of each species of Drosophila choosing a given yeast. Also shown are the ranges of the mean percentage for the six strains of each species of Drosphila used.

B. Adult choice experiments

As with the larvae the adults show preferences for different yeasts. These preferences are not so marked as in the larvae nor is there always a correspondence between the preferences of the adults and those of the larvae. However, although differences do occur between larval choices and adult choices it is not possible on the basis of these experiments to state that there is an over all difference in the pattern of larval preference when compared to the pattern of preference of the adults of the same species.

As with the larval choice experiments the results, shown in figure 4, give the mean percentage of flies selecting the various yeasts and also show the range of the percentages for the six strains of each species of Drosophila. A comparison of the six possible pairs of yeast is shown in table 4 in which the *t* values for the differences between pairs of yeasts are given.

In the experiment involving yeasts 5–16, *D. affinis* shows a preference for *S. fragilis* (16) over the other yeasts; this is significant at the 5% level when compared to *S. veronae* (15) and when compared to *Hanseniaspora uvarum* (14). *S. fragilis* appears also to be favored when compared to *S. dobzhanskii* (5) but this is not certain. The situation is quite different in experiments with larvae in which yeasts 5, 15 and 16 are about equally chosen, while 14 seems to be avoided. *D. miranda* adults show no particular preference when offered the yeasts 5, 14 and 15 but show significant preferences for these three yeasts when compared with 16. The larvae, however, show about equal preference for 5 and 16 when compared with 14 and 15. *D. persimilis* adults show a preference for *S. dobzhanskii* (5) which is similar to the pattern established by the larvae. *D. pseudoobscura* tends to prefer *S. dobzhanskii* but shows a more significant preference for the other three yeasts when compared with yeast 16. The larvae also tend to prefer *S. dobzhanskii* but they do not exhibit the same aversion for yeast 16 that is indicated by the adults.

In the second series of experiments

TABLE 4. *The statistical significance, as measured by the* t *values, of the observed differences in the attractivity of the different species of yeasts to adults of Drosophila used in the three series of experiments*

	Yeast:	5–14	5–15	5–16	14–15	14–16	15–16
Series I	Drosophila:						
	affinis	0.54	0.29	1.65	0.08	2.57	2.82
	miranda	0.59	1.36	3.84	1.54	2.78	3.38
	persimilis	3.37	1.64	3.69	0.72	1.14	1.04
	pseudoobscura	1.66	1.76	5.15	0.03	2.52	2.58
Series II		16–19	16–20	16–23	19–20	19–23	20–23
	affinis	0.92	2.84	4.27	1.67	2.07	0.77
	miranda	2.32	2.03	4.89	0.04	1.14	1.42
	persimilis	1.99	1.52	2.64	1.24	0.22	1.47
	pseudoobscura	3.66	1.39	1.30	2.51	1.50	1.06
Series III		23–194	23–197	23–201	194–197	194–201	197–201
	affinis	1.19	0.31	1.99	2.60	2.54	1.67
	miranda	0.37	0.14	0.71	0.82	1.39	0.99
	persimilis	3.82	1.09	2.36	0.97	5.40	2.64
	pseudoobscura	2.17	2.97	0.61	0.19	1.12	1.54
		$t_{.05} = 2.57$		$t_{.01} = 4.03$		$t_{.001} = 6.86$	

involving yeasts 16–23, *D. affinis* shows a reversal of its preference for *S. fragilis* (16) observed in the previous experiments. In this combination yeasts 20 and 23 are both preferred to 16. There is a similar indication with respect to yeast 19 which is not clearly differentiated by preference from yeast 16. This pattern of preference is generally the same in the experiments with the larvae of *D. affinis*. *D. miranda* continues in the second experiment not to favor *S. fragilis* (16). There is an indication of a slight preference for *Candida krusei* (23) over the other two species of Candida in this experiment. The larvae of *D. miranda* while showing a similar lack of preference for yeast 16, show slightly less preference for *Candida parapsilosis* (20) than did the adults. The adults of *D. persimilis* show greatest preference for yeast 23 and least preference for yeast 16. The larvae of this species show greatest preference for yeast 19 and least preference for yeast 16. *D. pseudoobscura* shows least preference for yeast 16 and greatest preference for yeast 19. The larvae, much more variable in their response than the adults, tend to prefer *Candida krusei* (23) and show least preference for yeast 16.

In the third experiment none of the species shows the pronounced preference for *Candida krusei* (23) which is exhibited by the larvae of all species. Indeed, with the exception of *D. pseudoobscura*, the adults tend slightly to prefer *Kloeckera apiculata* (201). *D. affinis* while indicating a preference for yeast 201 never reaches the 5% level of significance when comparison is made between yeast 201 and the other three yeasts. *D. miranda* adults show no preference for the four yeasts but the highest percentage of larvae choose yeast 201. *D. persimilis* shows least preference for yeast 194 and greatest preference for yeast 201. *D. pseudoobscura* changes the pattern by showing a greater preference for *Candida krusei* (23) but only a relatively slight preference over yeast 201.

C. Larval growth experiments

The results of this experiment are indicated in table 5. This experiment is concerned with the growth supporting properties of the various yeasts free of any substrate, which serves as a pabulum for the yeast itself.

The role of various substrates for yeast growth has not been investigated extensively with reference to their ability to support the growth of larvae. Wagner (1944) showed that cactus juice agar inoculated with certain yeasts could not support growth of larvae of *D. aldrichi*. Dudgeon (1954) has shown that a peptone agar substrate inoculated with certain yeasts failed to support growth of larvae of the *virilis* group species. It has been our experience that newly hatched larvae of *D. pseudoobscura* fail to develop on malt agar in the absence of yeast.

One yeast, *Hanseniaspora uvarum* (14), was not able to support growth when given to larvae as a washed suspension. This yeast, however, was able to support the growth of larvae of *D. pseudoobscura* when grown on sterile cream of wheat-molasses medium. It would seem, therefore, that the medium on which the yeast grows cannot be neglected in determining the growth supporting property of a yeast species. If, on the other hand, one uses a washed suspension of yeast, as in the present experiments, one is confronted

TABLE 5. *Percentages of larvae successfully reaching the adult stage when grown on washed yeast of a given species*

	D. persimilis		*D. pseudoobscura*	
Yeast	No. of eggs	% emerging	No. of eggs	% emerging
5	112	67.0	165	79.5
14	115	0.0	88	0.0
15	56	82.3	189	56.7
16	141	34.5	176	76.4
19	46	58.7	54	46.4
20	44	56.9	48	52.1
23	50	26.0	58	70.7
194	54	0.0	58	0.0
201	54	7.4	58	5.3

with the objection that the yeasts are not carrying out normal metabolism. The yeast may, therefore, be deficient in certain nutritional properties that would otherwise be present.

Several authors (Schultz, St. Lawrence and Newmayer 1946, Begg and Robertson 1950, and Hinton, Noyes and Ellis 1951) have shown that the nutritional requirements of Drosophila are extremely complex, consisting of as many as 18 amino acids and many vitamins and other nutritional factors. It was found that in the absence of certain factors as folic acid the growth rate was reduced. None of the various synthetic media, however, permits a growth rate equal to that of growth on baker's yeast.

It is interesting to compare the results shown in table 5 with the relative preference shown by the larvae of *D. pseudoobscura* and *D. persimilis* for the ten yeasts used in the three series of experiments shown in table 3. It can be seen that *D. persimilis* tends to prefer those yeasts that support growth. While yeast 197 was not tested in this experiment another strain of this same species (*Candida mycoderma*) was tested and failed to support growth. Thus, yeasts 14, 194, 197 and 201 are least preferred and apparently are least able to support growth of larvae. A similar trend can be seen in *D. pseudoobscura* with the exception of yeast 15 which does support growth but is near the bottom of the preference scale. Although this comparison is a very crude one, it does seem to bear out the idea that larvae of these two species prefer yeasts that support growth.

Discussion and Conclusions

The present experiments, as well as those of Lindsay (1958), show that the larvae of each species of Drosophila studied show preferences for different species of yeasts. The results indicate further that, at least with *D. pseudoobscura* and *D. persimilis*, the yeasts preferred by the larvae tend to be those that best support the growth of the larvae. Moreover, the choice of the yeasts exercised by the adults is not identical with the choice exhibited by the larvae.

The finding of larval preferences for different yeasts raises the question of what possible advantages may accrue to the species possessing such preferences. Lindsay has indicated that the preferred yeasts support a slower growth rate than those less preferred. In the attempts to develop a synthetic medium for Drosophila a slower growth rate has been used by Begg and Robertson (1950) as an indication of some nutritional deficiency in the medium. If we accept this criterion of nutritional deficiency, Lindsay's evidence would indicate that preferred yeasts are those that are less suitable for supporting the growth of the larvae. However, comparisons of data in tables 3 and 5 show that the larvae of *D. persimilis* and *D. pseudoobscura* prefer those yeasts that support growth rather than those yeasts that do not. On this basis it might be thought that the process of adapation through natural selection may result in larvae seeking out in the breeding sites the yeast that best supports growth. Indeed, in the laboratory, female flies often deposit eggs on the glass surfaces of the culture bottles and not on the medium in which the larvae develop. Thus, if the female flies do not lay eggs in the locations most suitable for the larvae, the ability to seek out suitable food would be advantageous to the larvae. In the third larval choice experiment using yeasts 23–201, yeast 23 was very much favored by larvae of all species. It is interesting to note, therefore, that the preferred yeast was the only species that supports the growth of the larvae. In the light of this experiment, it would be interesting to know (1) which, if any of these four yeasts would be chosen by a female fly when depositing eggs, and (2) how successfully larvae would grow on each of these yeasts when cultured on a yeast supporting medium free of other microorganisms.

The suggestion that the preferred food

sources of the adult Drosophila differ from those of the larvae was first put forth by Carson, Knapp and Phaff (1956). This suggestion was based on the fact that the yeasts isolated from slime fluxes, the only well established breeding site of *D. pseudoobscura* and *D. persimilis,* were different from those yeasts isolated from the crops of adults of these two species. In the present paper, it has also been shown that the larvae and adults do not have the same preferences when given the choice of the same group of yeasts. This agrees with the hypothesis of Carson, Knapp, and Phaff.

The most obvious advantage that may accrue to a species of Drosophila from a separation in the food niches of adults and larvae is lessening the intraspecific competition. But, if such a mechanism is needed to reduce intraspecific competition, is not some similar mechanism needed also to reduce interspecific competition for food?

There are many indications that competition between species of Drosophila is reduced by various means, in the Yosemite region of California where about ten species including *D. pseudoobscura, D. persimilis* and *D. miranda* coexist. Carson (1951) and Carson, Knapp and Phaff (1956) by their failure to find *D. azteca* breeding in the slime fluxes showed that the breeding sites of this species differ from those of *D. persimilis* and *D. pseudoobscura. D. azteca* is generally more difficult to raise in the laboratory on cream of wheat-molasses medium seeded with Fleischmann's yeast than are *D. pseudoobscura* or *D. persimilis.* A difference in the larval feeding habits is obvious, although *D. azteca* is closely related to *D. pseudoobscura* and *D. persimilis.* Furthermore, *D. azteca* is often more numerous at Mather than either of the latter species (Cooper and Dobzhansky, 1956). The difference in the breeding sites of *D. azteca* and the other two species means that one, and perhaps the most important source, of competition among these species is eliminated.

Pittendrigh (1958) has found that *D. pseudoobscura* is photonegative and more attracted to moisture than *D. persimilis.* He attributes this difference to the selective factors that operate in the habitats occupied by the two species. Since, as pointed out below, *D. pseudoobscura* occupies drier habitats than *D. persimilis* the photonegativity is correlated with water conservation. The flies seek out dark areas where the relative humidity is higher during the drier periods of the day when light intensity is high. The advantage to such a species of sensitivity to moisture is obvious. As pointed out by Dobzhansky and Epling (1944), *D. pseudoobscura* is more commonly found in the relatively warmer and drier parts of the distribution it shares with *D. persimilis.* The latter species is more frequently found in the cooler and moister parts of their range. In the Yosemite region (see Cooper and Dobzhansky, 1956) *D. pseudoobscura* can survive the hot, dry climate that is characteristic of the summers at elevations of 3,000 feet and lower. *D. persimilis* is not able to maintain a population of any size under these conditions. Its population size does, however, increase at these elevations during the cooler and more humid months of the year. *D. persimilis* is, however, more commonly found than *D. pseudoobscura* at elevations of 6,000 feet and above in the Yosemite region at all seasons. Pittendrigh (*op. cit.*) also believes that the habitats preferred by *D. persimilis* and *D. pseudoobscura* differ at Mather in the Yosemite region. The habitats referred to are the different types of woodland. *D. persimilis* is reported to be more common in the moister, cooler stands of *Libocedrus* and *Abies* and *D. pseudoobscura* is more common in the drier woodlands consisting mostly of *Quercus.* Competition between *D. pseudoobscura* and *D. persimilis* is further reduced by the time of day at which the adults are active. Dobzhansky *et al.* (1956) have shown that *D. persimilis* is a relatively more active feeder in the

morning while *D. pseudoobscura* visits traps more frequently in the early evening.

D. miranda is much less common than its sibling species *D. pseudoobscura* and *D. persimilis* in the Mather region. There are indications in Phaff *et al.* (1956) that the adults feed on yeasts different from those of *D. persimilis* whose geographic distribution it most closely parallels. Sokoloff (1955) has shown that the larvae of *D. miranda* are unable to survive when placed in the same culture tube with the larvae of *D. pseudoobscura* or of *D. persimilis*. The adult stage of *D. miranda* is more sensitive to high temperatures than are the adults *D. pseudoobscura* or *D. persimilis*. Although the relatively smaller population size of *D. miranda* makes the finding of its breeding sites less probable, it should be noted that *D. miranda* larvae have not been found in any of the breeding sites that have been found for other species of Drosophila in the Yosemite region.

There are, therefore, strong indications that factors exist that would tend to reduce competition between four of the species commonly found in the Yosemite region. It should be noted that these four species, *D. azteca, D. miranda, D. persimilis* and *D. pseudoobscura* are closely related. All belong to the *obscura* species group, and the latter three are sibling species.

The two other commonly occurring species of the Yosemite region (aside from the two cosmopolitan species *D. melanogaster* and *D. immigrans*) are *D. pinicola and D. occidentalis*. Both species are common at Mather. *D. occidentalis* is a fungus feeder but is also attracted to baits of bananas inoculated with yeast. It seems to prefer the moister areas about streams where, as indicated in Cooper and Dobzhansky (1956), it is very abundant indeed. *D. pinicola* is a sap feeder which is very difficult to raise in the laboratory (Patterson and Stone, 1952). Carson (1951) has found *D. pinicola* feeding on slime fluxes of *Quercus kelloggii* but has not found them breeding there. Carson *et al.* (1956) reared a few adults of *D. pinicola* from the fungus *Clavaria*.

In view of these various factors that could contribute to the reduction of competition between species, it is worth considering the preferences for various yeasts exhibited by the *obscura* group flies. According to the data now available, the food preferences of both adults and larvae differ relatively slightly in these closely related species. Dobzhansky *et al.* (1956) have shown that the food preferences of *D. azteca* are not the same as those of *D. pseudoobscura* and *D. persimilis*. But the food preferences of the latter two species appear to be quite similar.

In the present experiments, differences between species with respect to yeast preferences do exist. The larvae of *D. persimilis* in the first series of larval preference experiments are relatively more strongly attracted to yeast 16 than are the larvae of *D. pseudoobscura*. In the second series of this experiment *D. persimilis* larvae favor yeast 19 while *D. pseudoobscura* larvae favor yeast 23. How important these differences are in reducing competition between these two species is not known. The similarities in the larval choice experiment seem, however, more striking than the differences. In series I yeast 5 is the most preferred yeast for all four species. In series II yeasts 19 and 23 are the two preferred yeasts of all species of Drosophila. Even allowing for the differences in the reaction of *D. persimilis* and *D. pseudoobscura* to these two yeasts, the fact remains that they are the yeasts preferred to yeasts 16 and 20. In the third series yeast 23 is preferred by all species of Drosophila.

In the adult choice experiments differences are also shown by the species of Drosophila with respect to their preference for the same group of yeasts. In the first series *D. affinis* prefers yeast 16. This yeast is least preferred by the other species. In series II, *D. pseudoobscura* prefers yeast 19; the other species prefer yeast 23. In series III *D. pseudoobscura* prefers yeast 23; the other species prefer

yeast 201. There is less uniformity among adults than among larvae in preferences for a given group of yeasts. The only instance in the adult choice experiment of a uniform preference is in series II in which all four species prefer yeast 16 least.

The fact that the larvae of *D. pseudoobscura* and *D. persimilis* prefer the same yeasts (albeit to different degrees) requires some explanation. That the habitats of *D. pseudoobscura* and *D. persimilis* while over-lapping tend to be ecologically different has been shown by Dobzansky and Epling (1944), Dobzhansky *et al.* (1956) and Pittendrigh (1958). The factors cited by these authors, while not associated directly with food would nevertheless reduce competition also for food. It might be expected, under these conditions that selection would favor a preference for yeasts that would best support growth rather than to favor preferences that would mitigate an already reduced competition for food. This idea is supported by the evidence reported in the present paper. Furthermore, even at Mather where these two species are about equally common and quite numerous, there is little evidence to suggest that competition between larvae of these two species is greater than intraspecific competition would be. Carson (1951) has isolated larvae of both species from the same slime fluxes (the only well established site). But as pointed out by Sokoloff (1955) the number of larvae isolated from any single slime flux is usually very small. Other organisms within fluxes at Mather were generally not very numerous either. None of the fluxes observed at Mather (personal observation) supported the high density of beetle larvae observed in slime fluxes on a white oak (*Quercus alba*) found at Baldwin, N. Y. Here about 100 Drosophila of various species (none of them cosmopolitan) were collected in a single evening. Spieth (quoted by Patterson and Stone, 1952) also mentions a high density of other organisms in a flux on which he found *D. affinis* and other species feeding. In this flux apparently, the only one of the drosophilids that was breeding was *D. robusta*. Sokoloff (*op. cit.*) suggests that other organisms may interfere with the egg-laying process and with feeding by adults at slime fluxes.

Sokoloff (1955) has shown that when five larvae of *D. pseudoobscura* and five larvae of *D. persimilis* are placed in a vial containing 4 ml. of medium inoculated with yeast, the resulting competition causes effects no different from ten larvae of each species being raised separately under the same culture conditions. While this compatibility may not hold true for all species of yeast, it does indicate that the larvae can coexist with moderate crowding.

It may well be that in the temperate zone, unlike the conditions in the tropics, the variety of feeding and breeding sites is limited thus reducing the opportunity for greater specialization in closely related species occupying the same niche. In the Yosemite region where a considerable search for breeding sites has been made, larvae of sap feeding Drosophila have been found in four different types of breeding sites. These are slime fluxes of oak, slime flux of *Abies* (one pupa of *D. pseudoobscura* in 46 fluxes), sap exudations of aspen (*Populus*), and in the fungus *Clavaria*. Possible feeding sources are no more common. This is very unlike the situation in tropical regions. Dobzhansky and Pavan (1950) have shown that in Brazil a variety of fruits attract Drosophila and that the degree of discrimination for the various fruits is considerable. Da Cunha, Shehata and Oliveira (1957) and Dobzhansky and da Cunha (1955) have also shown that a considerable amount of preference is shown by Brazilian species of Drosophila for different species of yeasts. Burla (1955) has shown that in Africa 31 different species of Drosophila visited cut palm trees at different times after the cut was made. Burla believes that this was due to an alteration of the bait due to different stages in fermentation. (It might also be that the bait

contains different microorganisms at different times.) Burla also showed that considerable variation existed in the time of day that flies visited the baits, and also in the duration of the feeding periods.

In the temperate zone it would appear from the evidence presented in this paper that even allopatric species such as *D. affinis* do not differ greatly in their food preferences from closely related species living in quite different environments. Dobzhansky and da Cunha (1955) have shown that in Brazil geographically separated populations of the same species of Drosophila may have decidedly different preferences when offered the same group of yeasts. It would be interesting to see what preferences for yeast populations of *D. pseudoobscura* from different geographical regions would exhibit.

That the four closely related species of the Yosemite region of California are ecologically differentiated, there can be no doubt. Two of the species *D. miranda* and *D. azteca* seem to be most distinct in their feeding habits and in choice of breeding sites from *D. pseudoobscura* and *D. persimilis*. These latter two species do not seem to be well differentiated in feeding habits or choice of breeding sites. They are, however, differentiated ecologically in other ways. How these similarities and differences interact to permit their coexistence must await further study.

Summary

1. Experiments have been conducted to determine the feeding preferences of the larvae and the adults of *D. affinis*, *D. miranda*, *D. persimilis* and *D. pseudoobscura*. The flies or their larvae were offered choices among ten species of yeasts. These yeasts were originally isolated from the crops of these flies or from the areas in which the flies occur.

2. It has been found that larvae and adults of every species studied prefer certain yeasts to others. However, the preferences exhibited by the adults are not identical with those of the larvae of the same species.

3. It has been shown that larvae of *D. persimilis* and *D. pseudoobscura* do not develop equally well on the several yeasts used. There is evidence that the larvae show preferences for those yeasts on which they grow most successfully.

4. In the discussion the probable significance of the ecological differentiation of the four species of the *obscura* group of Drosophila has been pointed out.

5. It has been concluded that, in *D. persimilis* and *D. pseudoobscura*, selection has favored preferences for yeasts that best support growth rather than preferences that tend to differentiate the food niches of these two species. With other species a different situation is observed.

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THE BEHAVIOR OF CYTOPLASMIC POLLEN STERILITY IN POPULATIONS

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I. INTRODUCTION

Pollen sterility in plants is the most frequently observed character induced by the cytoplasm. The extensive material on this subject has been thoroughly reviewed by Rhoades (1955) and by Edwardson (1956). In almost all cases, disturbances in the development of pollen arise when particular genes are located in particular cytoplasms. This occurs mostly in crosses between different species or different local strains of the same species. The same phenomenon has been frequently observed in inbred lines of cultivated plants.

As an example, the classical case of pollen sterility arising in crosses between two "strains" of *Linum*, tall and procumbent, will be briefly reviewed. The case was originally described by Bateson and Gairdner (1921) and further analysed by Chittenden and Pellew (1927) and by Gairdner (1929). It appears likely that the two "strains" of *Linum* used in these experiments represent actually different species (Gajewski, 1937). If the strains tall and procumbent are crossed in both reciprocal directions, the F_1s are completely fertile. If the original female parent had been tall, the F_2 is also fertile. If, however, the original female parent had been procumbent, one fourth of the F_2 plants are pollen sterile, indicating Mendelian segregation. It is concluded that the original tall strain was homozygous for a recessive gene *f* which, if it becomes homozygous in procumbent cytoplasm, inhibits the formation of pollen. Since the original tall strain was fertile, the gene *f* does not induce pollen sterility in the cytoplasm of the tall strain. Furthermore, the original strain procumbent must have been homozygous for a dominant allele of *f* which does not lead to pollen sterility in either one of the two cytoplasms and may be called *F*. Pollen sterility in this cross arises, then, as a result of an interaction between the cytoplasm of the procumbent strain and the recessive gene *f*.

The genetic scheme implied in this interpretation can be applied to most cases of pollen sterility in plants, with the modification that in many instances more than one pair of alleles is involved. Genes which in combination with a particular cytoplasm give rise to particular characters, such as pollen sterility, have been designated as "plasmaempfindlich" by Michaelis. This term has been rendered in English as "plasmon sensitive" (Caspari, 1948) and "cytoplasmically sensitive" (Edwardson, 1956). It does not imply that there exist two categories of genes, sensitive and non-sensitive, but only that in a certain cross certain pairs of alleles interact differentially with the same cytoplasm. In principle it is assumed that probably every gene may appear sensitive to certain cytoplasms. Jones (1951, 1954) and his collaborators, starting out with cytoplasmically pollen sterile lines of cultivated plants, particularly maize, found that in certain crosses to other lines pollen fertility was restored, due to the action of dominant genes present in the second line. They called these genes "restorer genes." It

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should be noted that the terms "restorer genes" and "plasmon sensitive genes" are complementary terms. In the quoted example from *Linum*, *F* would be called a "restorer gene" in the sense of Jones, its allele *f* a "plasmon-sensitive gene" in the sense of Michaelis.

In the present paper, a model for the behavior of genes and cytoplasms in a population will be developed. It is assumed that the character, pollen sterility, is due to the interaction of Mendelian genes and a maternally transmitted cytoplasm, as shown in the example from *Linum*.

II. Pollen Sterility—No Mutations

We consider a large hermaphrodite population in which occur two types of cytoplasm, Φ, φ, and a locus with two alleles, F, f. Individuals of the types

$$\Phi FF,\ \Phi Ff,\ \Phi ff, \qquad \varphi FF,\ \varphi Ff,\ \varphi ff$$

exist with frequencies given, respectively, by

$$\Phi_1,\ \Phi_2,\ \Phi_3, \qquad \varphi_1,\ \varphi_2,\ \varphi_3.$$

We will write:

$$\Phi_1 + \Phi_2 + \Phi_3 = \alpha$$
$$= \text{frequency of } \Phi \text{ cytoplasm}$$
$$\varphi_1 + \varphi_2 + \varphi_3 = \beta$$
$$= \text{frequency of } \varphi \text{ cytoplasm}$$

so that $\alpha + \beta = 1$.

We now suppose that a round of random mating takes place in this population on the further assumptions that

(i) the number of offspring from all matings is equal,
(ii) all individuals yield fertile ova,
(iii) all individuals yield completely fertile pollen except the Φff and φff types,
(iv) the fractions of fertile pollen from types Φff and φff are, respectively, ν_1 and ν_2,
(v) the cytoplasm of the offspring is identical with that of the parent ovum.

The gene frequencies in the two cytoplasmic groups are

$$p = \text{frequency of F in } \Phi \text{ cytoplasm},$$
$$= \frac{2\Phi_1 + \Phi_2}{2\alpha},$$
$$q = \text{frequency of f in } \Phi \text{ cytoplasm},$$
$$= \frac{\Phi_2 + 2\Phi_3}{2\alpha},$$
$$r = \text{frequency of F in } \varphi \text{ cytoplasm},$$
$$= \frac{2\varphi_1 + \varphi_2}{2\beta},$$
$$s = \text{frequency of f in } \varphi \text{ cytoplasm},$$
$$= \frac{\varphi_2 + 2\varphi_3}{2\beta}.$$

Thus $p + q = 1$, $r + s = 1$.

The overall gene frequencies are

$$P = \text{frequency of F}$$
$$= \frac{2\Phi_1 + \Phi_2 + 2\varphi_1 + \varphi_2}{2} = p\alpha + r\beta,$$
$$Q = \text{frequency of f}$$
$$= \frac{\Phi_2 + 2\Phi_3 + \varphi_2 + 2\varphi_3}{2} = q\alpha + s\beta,$$

so that $P + Q = 1$.

In this section it will be assumed that there is no mutation so that this population produces ova of the types

$$\Phi F \quad \Phi f \qquad \varphi F \quad \varphi f$$

with frequencies

$$p\alpha \quad q\alpha \qquad r\beta \quad s\beta, \qquad (1)$$

adding to unity.

The cytoplasmic origin of the pollen is, by assumption, irrelevant. The relative proportions of F and f in fertile pollen are respectively:

$$2\Phi_1 + \Phi_2 + 2\varphi_1 + \varphi_2,$$
$$\Phi_2 + \varphi_2 + 2\nu_1\Phi_3 + 2\nu_2\varphi_3$$

or, in fractions adding to unity

$$kP, \quad 1 - kP, \qquad (2)$$

where k is the reciprocal of the fraction of pollen that is fertile, i.e.

$$\frac{1}{k} = \frac{1}{2}(2\Phi_1 + \Phi_2 + 2\varphi_1 + \varphi_2 + \Phi_2 + \varphi_2 + 2\nu_1\Phi_3 + 2\nu_2\varphi_3).$$

In the progeny, the various proportions defined above will be denoted by the same symbols with a prime added. Thus, for example, Q′ denotes the frequency of f in the next generation. Thus the relative proportions of the individuals in the next generation are

$$\left.\begin{aligned} \Phi'_1 &= kp\alpha P \\ \Phi'_2 &= p\alpha(1 - kP) + kq\alpha P \\ \Phi'_3 &= q\alpha(1 - kP) \\ \varphi'_1 &= kr\beta P \\ \varphi'_2 &= ks\beta P + r\beta(1 - kP) \\ \varphi'_3 &= s\beta(1 - kP). \end{aligned}\right\} \quad (3)$$

It follows immediately that $\Sigma\Phi'_i = \alpha' = \alpha$, $\Sigma\varphi'_i = \beta' = \beta$, i.e., the relative proportions of the two types of cytoplasm remain constant as is clear from the model. The gene frequencies change however and to study them we obtain, by the routine approach,

$$p' = \frac{2\Phi'_1 + \Phi'_2}{2}, \qquad = \frac{p}{2} + \frac{kP}{2}, \quad (4)$$

after using the expression (3) for Φ'_1, Φ'_2 and simplifying. However it is more helpful to derive (4) as follows. The gene F in an individual with cytoplasm Φ has an equal chance of coming from the parental ovum or pollen. Since the parental egg must be Φ type, the chance of it containing F is, by (1), p. By (2) the chance of fertile pollen containing F is kP. Putting these results together, (4) is obtained. Similarly we may show that

$$r' = \frac{r}{2} + \frac{kP}{2}, \quad (5)$$

$$P' = \frac{k+1}{2}P \quad (6)$$

Equations (4) and (5) combine to give

$$p' - r' = \frac{p - r}{2}. \quad (7)$$

This means that, if ever p = r, this equality will hold thereafter. If $p \neq r$, the difference between them is reduced by a factor 1/2 at each generation so they rapidly become equal. In (6), since 1/k is between 0 and 1, the factor (k + 1)/2 is greater than unity. Hence this equation shows how the frequency of F *increases*. The rate of increase of F depends on the value of k, i.e., on the value of

$$\frac{1}{k} = 1 - (1 - \nu_1)\Phi_3 - (1 - \nu_2)\varphi_3. \quad (8)$$

As f is eliminated, Φ_3 and φ_3 will decrease so that k decreases towards unity. Hence (k + 1)/2 decreases to unity. This has the effect of decelerating the progress of P to unity. Thus the difference between p and r should become unobservably small well before the disappearance of f, when the equality is trivially true.

To examine the ultimate fashion in which P tends to unity, we will assume that $p = r = P = 1 - \delta$ where $\delta = Q = q = s$ is small. Then Φ_2 and φ_2 will be of order δ and Φ_3 and φ_3 of order δ^2 in smallness. We will neglect all expressions of smaller order than δ^2. From (8) we may write, to this order of approximation,

$$k = 1 + (1 - \nu_1)\Phi_3 + (1 - \nu_2)\varphi_3, \quad (9)$$

i.e.,

$$\frac{k+1}{2} = 1 + \frac{1}{2}(1 - \nu_1)\Phi_3 + \frac{1}{2}(1 - \nu_2)\varphi_3. \quad (10)$$

Substituting, (10) in (6), we have

$$p' = 1 - \delta + \frac{1}{2}(1 - \nu_1)\Phi_3 + \frac{1}{2}(1 - \nu_2)\varphi^3. \quad (11)$$

Substitution of (9) in the equation (3) for Φ'_3 and φ'_3 leads to

$$\left.\begin{aligned}\Phi'_3 &= \alpha\delta^2\\ \varphi'_3 &= \beta\delta^2\end{aligned}\right\} \qquad (12)$$

so that (11) becomes

$$P'' = 1 - \delta' + \frac{1}{2}[(1-\nu_1)\alpha + (1-\nu_2)\beta]\delta^2$$

or

$$\delta'' = \delta' - \frac{1}{2}[(1-\nu_1)\alpha + (1-\nu_2)\beta]\delta^2, \qquad (13)$$

a non-linear recurrence relation connecting the frequencies of f at three successive generations. The larger the quantity

$$(1-\nu_1)\alpha + (1-\nu_2)\beta \qquad (14)$$

the more rapidly will the gene f be eliminated. In the simplest case where only φff individuals are affected and completely sterile, i.e., $\nu_1 = 1$, $\nu_2 = 0$, this quantity is β. The solution of (13) to order δ^2 is clearly

$$\delta' = \delta - \frac{1}{2}[(1-\nu_1)\alpha + (1-\nu_2)\beta]\delta^2, \qquad (15)$$

from which we find the simple approximation,

$$\frac{1}{\delta'} = \frac{1}{\delta} + \frac{1}{2}[(1-\nu_1)\alpha + (1-\nu_2)\beta]. \qquad (16)$$

Thus while the decrement in δ per generation is not a simple quantity, the increase in $1/\delta$ per generation is the constant $\frac{1}{2}[(1-\nu_1)\alpha + (1-\nu_2)\beta]$.

This completes the analysis of pollen sterility in the absence of mutation. The results are that the gene frequencies in the two cytoplasmic types tend rapidly to equality while the elimination of the f-gene proceeds in arithmetic steps of $\frac{1}{2}[(1-\nu_1)\alpha + (1-\nu_2)\beta]$ in the scale of $1/\delta$. The relative proportions of the two cytoplasms remain unaltered.

III. Pollen Sterility with Mutation

Into the discussion of II, we now introduce mutation. There are various possibilities. The simplest case of interest would be a mutation F → f which might overcome the selection pressure against f. A uniform mutation rate μ will be assumed. Thus the relative proportions among ova, formerly given by (1), is now modified to

ΦF	Φf
$p\alpha(1-\mu)$	$q\alpha + \mu p\alpha$
φF	φf
$r\beta(1-\mu)$	$s\beta + r\beta\mu$

(17)

To find the new proportions of F and f among fertile pollen, we now have the relative fractions (neglecting μ^2)

$$\begin{aligned}&2\Phi_1(1-2\mu) + \Phi_1 2\mu + 2\varphi_1(1-2\mu)\\ &\quad + \varphi_1 2\mu + \Phi_2(1-\mu) + \varphi_2(1-\mu),\\ &\qquad \Phi_1 2\mu + \Phi_1 2\mu + \Phi_2(1+\mu)\\ &\qquad\quad + \varphi_2(1+\mu) + 2\nu_1\Phi_3 + 2\nu_2\varphi_3.\end{aligned}$$

The fraction of pollen that is fertile is the same as before, $1/k$, so that proportions of F and f among fertile pollen is

$$kP(1-\mu), \quad 1 - kP(1-\mu). \qquad (18)$$

Thus the relative proportions of the individuals in the next generation is now, according to the method of II(3),

$$\begin{aligned}\Phi'_1 &= p\alpha(1-\mu)kP(1-\mu)\\ &= kp\alpha P(1-\mu)^2 = kp\alpha P(1-2\mu)\\ \Phi'_2 &= p\alpha(1-\mu)[1-kP(1-\mu)]\\ &\quad + (q\alpha+\mu p\alpha)kP(1-\mu)\\ \Phi'_3 &= (q\alpha+\mu p\alpha)[1-kP(1-\mu)] \qquad (19)\\ \varphi'_1 &= r\beta(1-\mu)kP(1-\mu)\\ &= kr\beta P(1-\mu)^2\\ \varphi'_2 &= r\beta(1-\mu)[1-kP(1-\mu)]\\ &\quad + (s\beta+r\beta\mu)kP(1-\mu)\\ \varphi'_3 &= (s\beta+r\beta\mu)[1-kP(1-\mu)]\end{aligned}$$

The change per generation in the fraction of Φ-individuals carrying F is now

governed by the analogue of II(4)

$$p' = \frac{1}{2}p(1-\mu) + \frac{1}{2}kP(1-\mu)$$

$$= \frac{(1-\mu)}{2}(p + kP). \quad (20)$$

Similarly

$$r' = \frac{1}{2}r(1-\mu) + \frac{1}{2}kP(1-\mu)$$

$$= \frac{(1-\mu)}{2}(r + kP). \quad (21)$$

Combining (20) and (21)

$$p' - r' = \frac{1-\mu}{2}(p - r), \quad (22)$$

so that p and r tend to equality slightly faster than before.

It is clear in this case that α and β are again constant and that an equilibrium will be reached. To find the equilibrium frequencies we set $p' = p$, etc. Thus, from (22), $p = r$, and from (19),

$$\frac{1+\mu}{2}p = \frac{k}{2}P \quad (23)$$

But if $p = r$, $P = \alpha p + \beta r = p$; therefore

$$k = 1 + \mu.$$

But, from the definition of k, we have II(8) which with (23) yields

$$\mu = (1-\nu_1)\Phi_3 + (1-\nu_2)\varphi_3. \quad (24)$$

Setting, in the two relevant equations of (19), $\Phi'_3 = \Phi_3$, $\varphi'_3 = \varphi_3$ and introducing the result in (24), we have as an equation for the equilibrium value of p, $\mu = \alpha(1-\nu_1)(q + \mu p)q + \beta(1-\nu_2) \times (q + p\mu)q$, i.e.,

$$\mu = [\alpha(1-\nu_1) + \beta(1-\nu_2)](q + \mu p)q.$$

Yielding the approximate solution

$$q = \sqrt{\frac{\mu}{[\alpha(1-\nu_1) + \beta(1-\nu_2)]}}. \quad (25)$$

The gene frequency (25) is in agreement with the results of part II. If $\mu = 0$ in (25), $q = 0$, as found before. Furthermore when $\alpha(1-\nu_1) + \beta(1-\nu_2)$ is large, q is small; whereas in II, large values of $\alpha(1-\nu_1) + \beta(1-\nu_2)$ were shown to mean rapid elimination of f.

IV. Egg Sterility—No Mutation

We turn now to egg sterility and suppose that φff individuals have sterile eggs. Using the above notation, the relative frequencies of fertile eggs of types

$$\Phi F, \ \Phi f \qquad \varphi F, \ \varphi f$$

are

$$p\alpha \quad q\alpha \qquad r\beta \quad \frac{\varphi_2}{2}$$

while, for pollen of types

$$F \qquad f$$

they are

$$P \qquad Q.$$

Since we are assuming that the egg determines the cytoplasm of the offspring, the relative frequencies of the two cytoplasmic types are

$$\Phi : h\alpha \qquad \varphi : h\left(r\beta + \frac{\varphi_2}{2}\right) \quad (26)$$

then

$$\frac{1}{h} = \alpha + r\beta + \frac{\varphi_2}{2} \quad (27)$$

$$= \text{fraction of fertile eggs.}$$

Thus

$$\alpha' = h\alpha \quad (28)$$

so that, since $h \geq 1$, the fraction of Φ cytoplasm increases, presumably to unity. To study the gene frequencies we observe that, in Φ cytoplasm, F occurs with frequency

$$p' = \frac{p}{2} + \frac{P}{2} = \frac{p+P}{2} \quad (29)$$

and, in φ cytoplasm, f occurs with frequency

$$r' = \frac{1}{2}\frac{r\beta}{r\beta + \frac{\varphi_2}{2}} + \frac{1}{2}P. \quad (30)$$

From these equations it follows again that

$$P' = \frac{h + 1}{2} P \qquad (31)$$

so that the frequency of F rises. Also,

$$p' - r' = \frac{1}{2}\left(p - r\frac{h\beta}{\beta'}\right). \qquad (32)$$

From (27) and the above results h tends to unity and, since $\beta' = h\beta + (1 - h)$, $h\beta$ tends to β' so that p' tends to r' as before.

To complete the discussion, we need to consider the case where

$$p = r = P = 1 - \delta,$$
$$\alpha = 1 - \Delta,$$

where δ, Δ are small. Until this case has some biological relevance, this work will be deferred.

V. Discussion

It has been suggested (Caspari, 1948; Jones, 1951, 1954) that cytoplasmic pollen sterility may act as a partial crossing barrier between geographical races and subspecies and has therefore importance for the origin of species. The considerations offered in this paper do not lend support to this idea. They show that if two strains differing in cytoplasma and plasmon sensitive genes are brought together, a unified Mendelian population will result, in which the frequencies of the genes involved in the production of pollen sterility will depend on the selective action of the cytoplasm. This is in agreement with considerations on zygote mortality due to incompatibility of two cytoplasms, as found in mosquitoes (Caspari and Watson, 1959). It is hard to see, on the basis of what is known about the action and behavior of cytoplasmic factors, how the presence of two different plasmons could result in the formation of two separate populations showing partial sexual isolation.

The model can account, on the other hand, for a number of characteristics frequently found in experiments involving cytoplasmic inheritance. In a large proportion of the cases described, e.g., pollen sterility in plants and lethality of male embryos in Drosophila, viability or fertility of the male sex are affected. It has been shown in the model, that the relative proportion of the two cytoplasms will remain constant in cases of cytoplasmic pollen sterility. Since the cytoplasm of the zygote is derived from the female parent only, effects on male viability or fertility will not lead to selection against the cytoplasm involved. The relative frequencies of two cytoplasms in a population will be acted upon by selection only according to their effects on the viability and fertility of the females.

On the other hand, the model demonstrates a selective effect on plasmon-sensitive alleles so that they will become eliminated in the course of time. This may account for the frequency with which cytoplasmic pollen sterility appears in crosses between species and local varieties of the same species from widely separated localities. In a population, a particular cytoplasmic condition will become established, and the genotypes of the population will be selected in such a way as to produce the fertile phenotype within this cytoplasm. In *Capsicum*, e.g., most wild populations seem to carry a restorer gene, while its plasmon-sensitive allele is widespread in cultivated varieties (Peterson, 1958). Nevertheless, in some wild populations the plasmon-sensitive allele has been found in this plant. In general, it has been found that different races and species may differ in their plasmons, but that their genotypes will usually be adjusted by selection in such a way as to produce pollen fertility. Only when two of these populations are crossed will a plasmon-sensitive gene come to lie in a cytoplasm with which it will produce pollen sterility. It should be added that the model presented has been restricted to the action of one pair of alleles, and

that the consequences of polygenic effects on cytoplasmic pollen sterility remain to be worked out.

A further consequence of the model presented is the possibility that in one and the same population two different plasmons may be present. This will be possible only if they have the same selective values in females. In this case, the allele inducing pollen sterility in combination with one of the cytoplasms will be eliminated from the population. The cytoplasmic differences will therefore not become phenotypically apparent in matings within the population, and can only be demonstrated in crosses to different strains or species which contain the allele involved. Such a situation has been described for the Insel Candidum strain of *Epilobium hirsutum* (Michaelis and Bakker, 1948, p. 397).

Among the few cases in which pollen sterility is dependent on the cytoplasm alone without the participation of nuclear genes are the cases of gynodioecy in *Cirsium* and *Satureia* described by Correns (1928). In this case populations consist of both hermaphroditic and pollen-sterile (female) plants, transmitting the sexual state from the maternal parent to all the offspring. Lewis (1941) has shown that pollen sterility induced by genes would require the genes involved to have at least twice the adaptive value of their pollen-fertile alleles in order to persist in the population, while for cytoplasmically induced pollen sterility any small selective advantage would be sufficient. Lewis suggests that gynodioecy in these plants is primarily an outbreeding mechanism, and that the selective advantage of the offspring of pollen-sterile plants may be due to the higher number of heterozygotes in their offspring due to the enforced pollination from other individuals. Lewis recognizes that actually gynodioecy of this kind can only persist in a population, if the pollen-sterile plants have a selective advantage in large populations, but are at a disadvantage in small populations. Similar conclusions have been drawn by Watson (1960) for the male killing cytoplasmic conditions in Drosophila, and may well constitute a prerequisite for the persistence of two different cytoplasms in the same population. If gynodioecism as an outbreeding mechanism is of advantage for a species, it can only be established in a population which is homozygous for a plasmon-sensitive gene. If a restorer gene was present in the population, it would replace its plasmon-sensitive allele, independently of selective advantages of the cytoplasm.

Michaelis (1958) has pointed out that the genome and the plasmon of any naturally occurring organism form a coordinated system. The model presented in this paper indicates a selective mechanism by which such a coordination may be achieved in evolution. The cytoplasm, as pointed out by Michaelis, differs from the genome by the fact that apparently numerous cytoplasmic components of the same type are present in each cell. Furthermore, there is a tendency in oogamous species to transmit the cytoplasm in bulk through the female parent only. In sexual reproduction, the plasmon does consequently not show recombination, although recombination of cytoplasmic components is possible in mitotic cell division. The lack of recombination of the plasmon in sexual processes renders the analysis of the plasmon difficult. It has the consequence that selection would be expected to act primarily on the plasmon as a whole, whereas a large variety of combinations of genes are available for the action of selection. Nevertheless, it has been frequently observed that cytoplasmic differences between local varieties of the same species exist. The origin of these differences constitutes an interesting problem in the study of evolution.

VI. Summary

A mathematical model has been developed for a population of plants in which pollen sterility is dependent on a

cytoplasmic factor and one pair of Mendelian alleles. The following conclusions can be drawn:

1. The ratio of the two cytoplasms in the population will remain constant.
2. The relative frequencies of the two alleles in the two cytoplasms will tend to equality.
3. The plasmon-sensitive allele involved in the production of pollen-sterility will be selected against and be eliminated.
4. The equilibrium conditions are given for the case that selection against a plasmon-sensitive gene is counteracted by mutation to the plasmon-sensitive allele.
5. Female sterility induced by interactions of a cytoplasmic factor and a nuclear gene leads to selection against the cytoplasm and the plasmon-sensitive gene, and to the elimination of both.

The bearing of the model on actual observations with cytoplasmic pollen sterility is discussed.

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LATITUDINAL VARIATIONS IN ORGANIC DIVERSITY

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"Animal life is, on the whole, far more abundant and varied within the tropics than in any other part of the globe, and a great number of peculiar groups are found there which never extend into temperate regions. Endless eccentricities of form and extreme richness of color are its most prominent features, and these are manifested in the highest degree in those equatorial lands where the vegetation acquires its greatest beauty and its fullest development." Thus wrote A. R. Wallace (1878). His remarks apply equally to the vegetable and animal kingdoms, to the terrestrial realm and to at least the surficial parts of the oceans. Surely this correlation of floral and faunal diversity with latitude is one of the most imposing biogeographic features on earth. On the one hand, its existence poses large-scale problems in evolution. On the other, it offers a potential tool to the geologist-paleontologist who attempts to wring patterns of earth history out of the fossil record.

The following pages serve to review some previously known latitudinal gradients in organic diversity, to describe quantitatively gradients in molluscan diversity along North American shores, and to inquire into the origin of these patterns.

SOME EXAMPLES OF DIVERSITY GRADIENTS

Terrestrial gradients

The overwhelming variety of trees in tropical rain forests has impressed northern travellers, from the early navigators on, and offers a striking contrast to the solid stands of timber to be found in the austral and boreal regions. This floral diversity gradient is widely recognized by botanists.

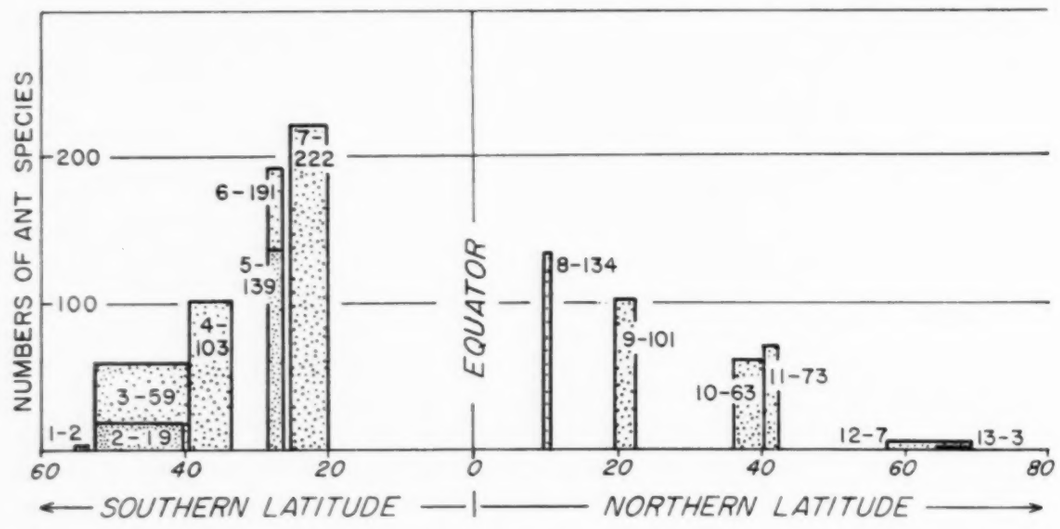

FIG. 1. Northern and southern diversity gradients in ants. 1—Tierra del Fuego; 2—Patagonia, humid western side; 3—Patagonia, as a whole; 4—Buenos Aires, Argentina; 5—Tucuman, Arg.; 6—Missiones, Arg.; 7—Sao Paulo, Brazil; 8—Trinidad; 9—Cuba; 10—Utah, USA; 11—Iowa, USA; 12—Alaska as a whole; 13—Alaska, arctic part. After Kusnezov (1957).

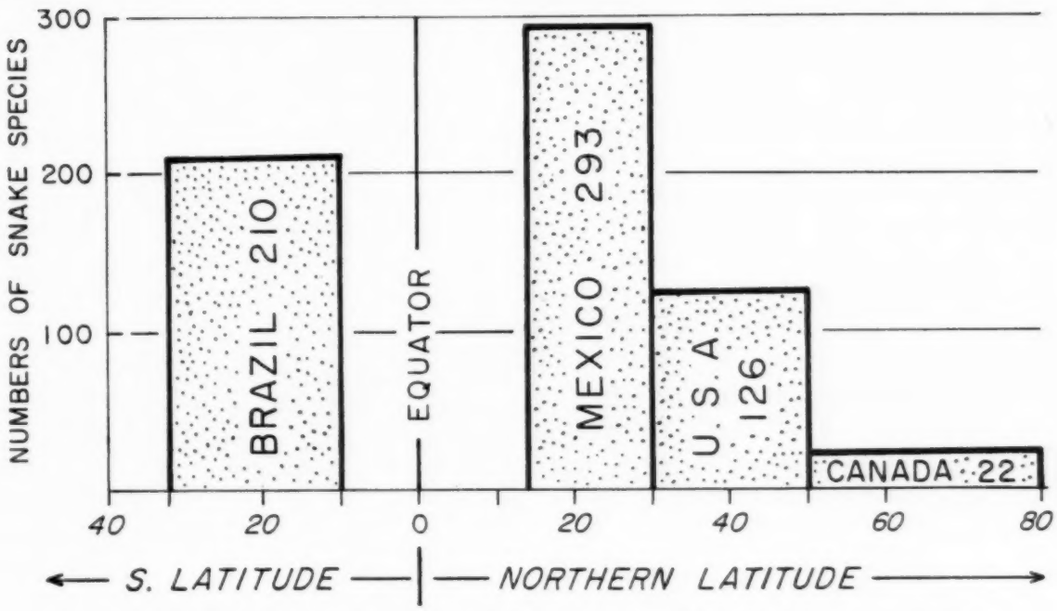

FIG. 2. Diversity gradient in American snakes. After Dobzhansky (1950, credited to Bogert).

Kusnezov (1957) compared ant diversity in areas of different latitudes, and his findings for the Americas are summarized in figure 1. In a stimulating paper dealing with the general problem of tropical diversity, Dobzhansky (1950) published figures on diversity gradients in American snakes (fig. 2) and birds (fig. 3). Distribution of snake species within Argentina follows a striking di-

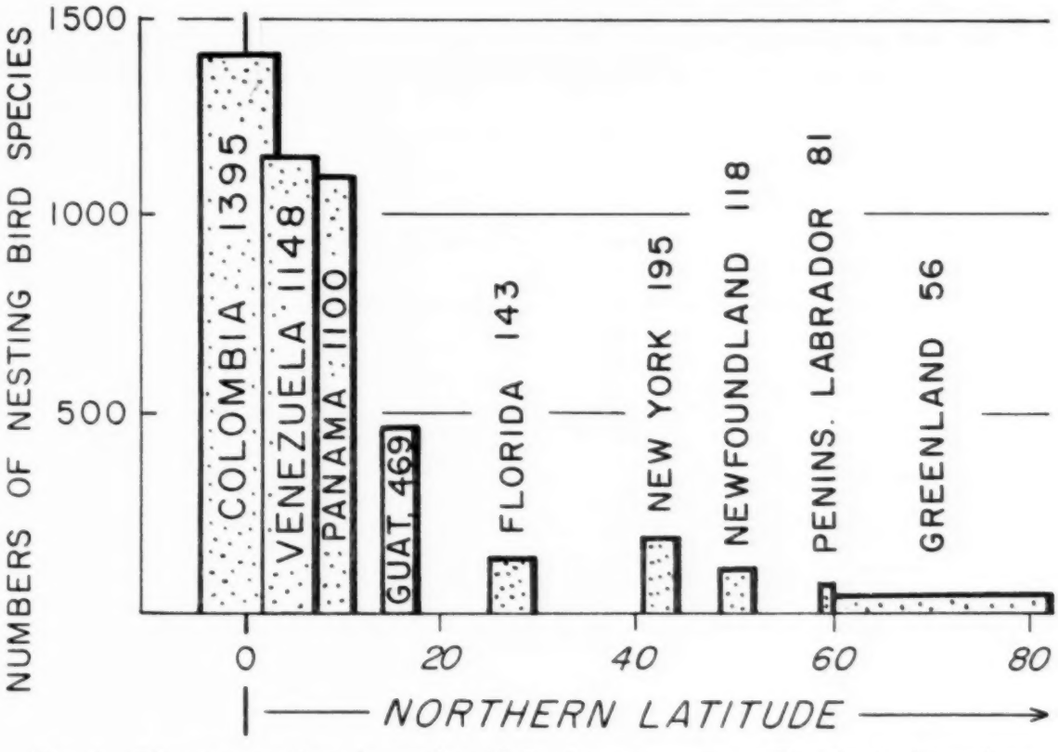

FIG. 3. Diversity gradient in nesting birds, from equator to Greenland. Data from Dobzhansky (1950, credited to Mayr).

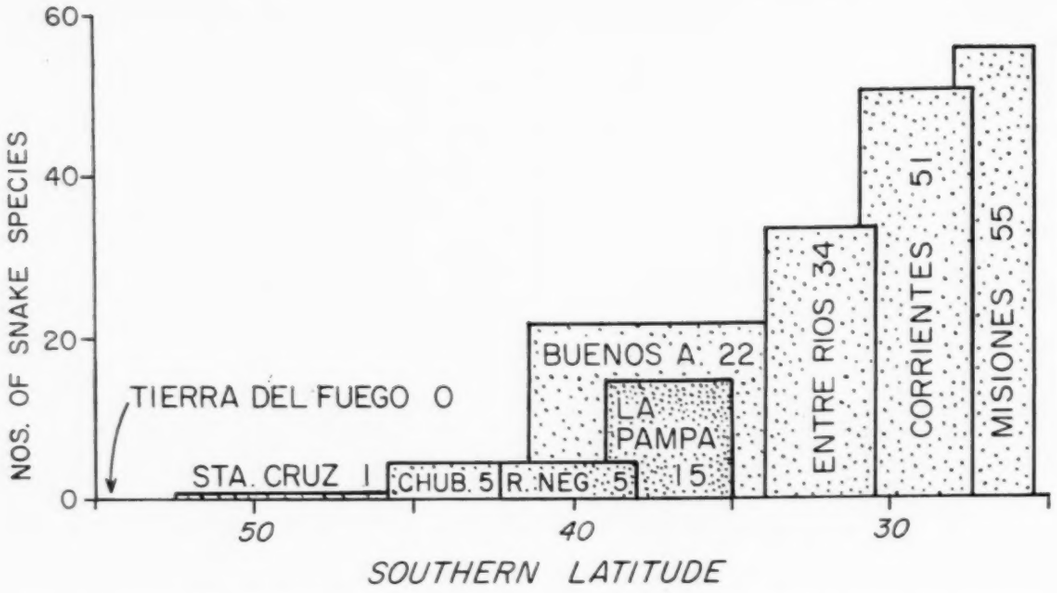

FIG. 4. Diversity gradient shown by numbers of snake species reported from the various provinces of Argentina. Data from Série (1936).

versity gradient described by Série (1936) (fig. 4). Bourlière (1957) shows comparisons of animal diversity between France and Barro Colorado Island in Panama.

Darlington (1957) does not deal with this problem as such, but points out repeatedly that amphibians, reptiles, birds and mammals are more diversified in the tropics than in higher latitudes; while the abundant figures provided by him are not readily plotted in diversity gradients, they illustrate the general principle.

Marine gradients

Any beachcomber who strays from northern shores into the tropics is im-

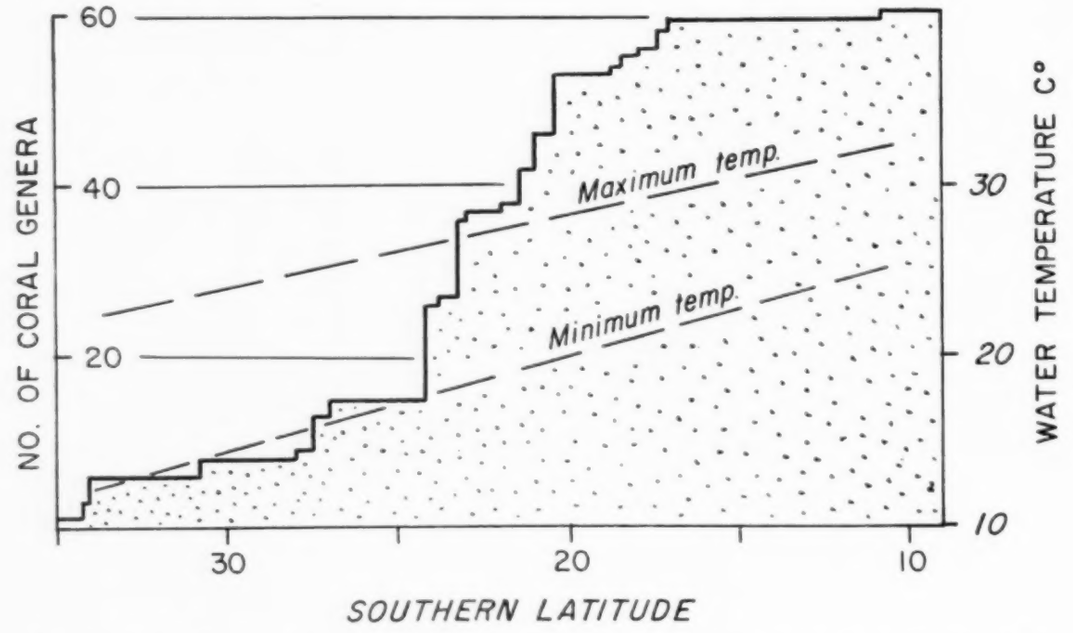

FIG. 5. Diversity gradient of coral genera along great Barrier Reef of Australia, after Wells (1956).

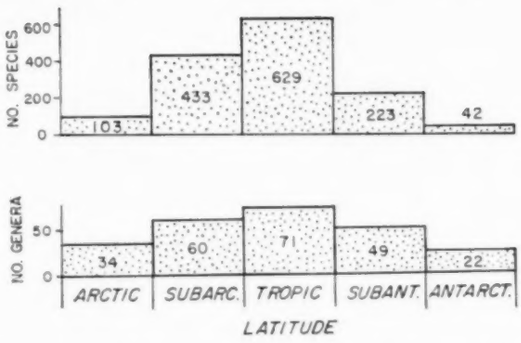

FIG. 6. Specific and generic diversity gradients in tunicates. After Hartmeyer (1909).

pressed with the greater variety of shells cast up on the shores there. Among general text and reference books the phenomenon has been noted, among others, in Hesse (1924) and Hesse, Allee, and Schmidt (1937), as well as by Thorson (1957).

Few if any groups of animals illustrate the principle more vividly than do the corals, with their bewildering variety of reef-building types in suitable tropical habitats, contrasted with a handful of solitary or at best bank-forming species in cold waters. Wells (1956) has studied the distribution of coral genera along the Great Barrier Reef of Australia, and his findings are summarized in figure 5. Some 60 genera of corals coexist in the northern part of this great reef belt, around lat. 9° S, and from this maximum the number dwindles to a single genus at the south end (35° S).

Hartmeyer (1919) illustrated similar diversity gradients in tunicates (fig. 6). Thorson (1952, 1957) shows similar gradients for amphipods, nudibranchs, and crabs (fig. 7), and Brodskij (1959) has illustrated the same principle in pelagic calanid crustaceans of the surficial water masses (fig. 8).

On the other hand, Thorson has shown that such gradients are not universal. As shown in figure 7, they are not exhibited by such burrowing groups as soft-bottom ophiuroids, holothuroids, cephalaspids and cumacids. These groups show little diversity anywhere. This difference between epifaunal or pelagic and infaunal animals is particularly well shown by Thorson's comparison of prosobranch gastropods (fig. 9). Taking prosobranchs as a whole, and comparing areas of equal size, tropical faunas show approximately five times as many prosobranch species as do arctic ones. Throughout this range, the great majority of prosobranchs is epifaunal. However, the prosobranch family Naticidae is largely restricted to infaunal soft-bottom dwellers, and the naticids show only slightly more diversity in the tropics than in high latitudes.

Some groups of organisms are more diverse in the temperate latitudes than in the tropics. This appears to be true for certain groups of algae. No actual figures on diversity are available to me, but from Tilden (1937) one would gather that

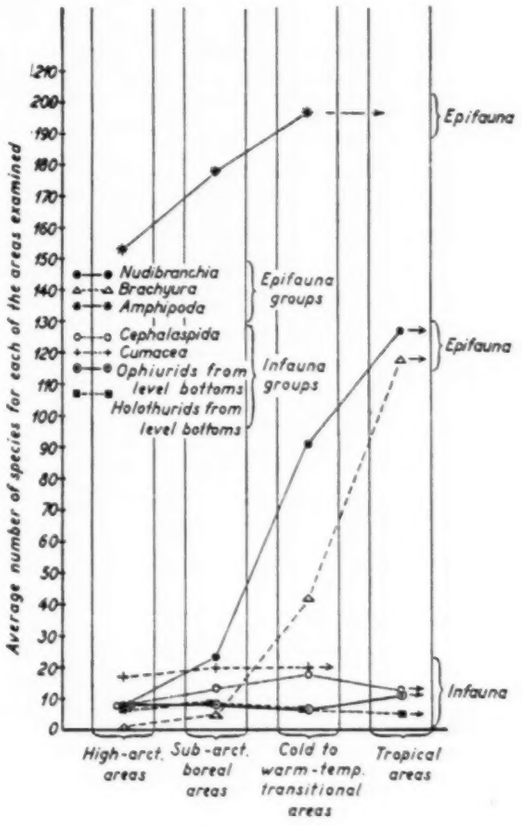

FIG. 7. Average number of species of different groups of bottom invertebrates from equal-sized coastal areas in different latitudes. Only depths from the shore to 300 m have been regarded, and all pelagic and parasitic species have been neglected. From Thorson (1952 and 1957).

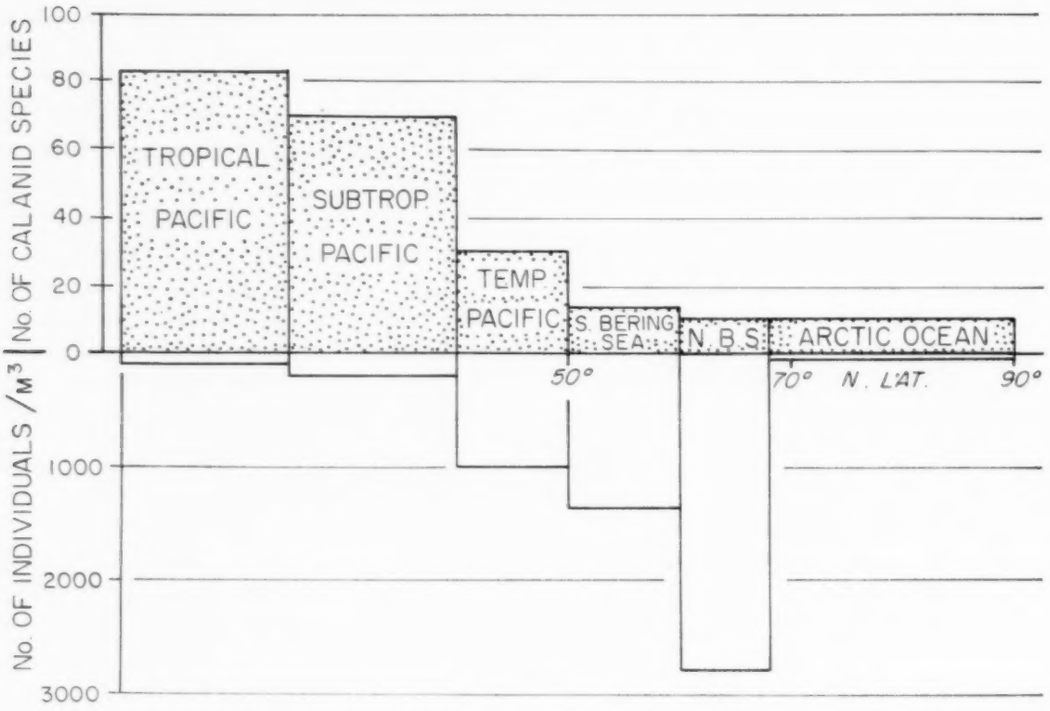

FIG. 8. Diversity gradient in calanids (pelagic crustacea) from the upper 50 meters. Abundance of individuals per cubic meter (lower graph) shows a strikingly divergent pattern. After Brodskij (1959).

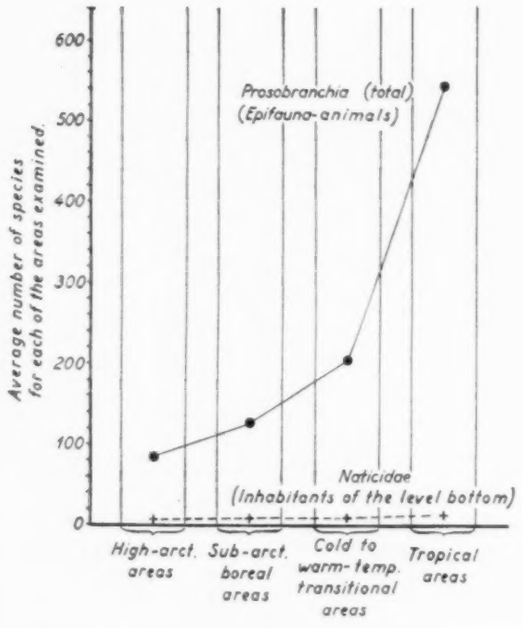

FIG. 9. Average number of prosobranch species from equally large coastal areas in different latitudes. Whereas the prosobranchs as a whole, a mainly epifaunal group, show a striking diversity gradient, the family Naticidae, mainly infaunal, does not. From Thorson (1952 and 1957).

whereas the green and the bluegreen algae are most diversely developed in the tropics, the red algae and kelps reach their acmes in the temperate zones.

Surely many similar examples of distribution, showing no correlation of diversity and latitude, or even "reversed" gradients from high diversity in temperate latitudes to little diversity in the tropics, could be found in various branches of the plant and animal kingdoms. They appear to be, however, no more than exceptions which prove the rule.

THE RELATION OF GRADIENTS TO CLIMATE

Within the tropical belt, similar gradients mark the passage from hot lowlands into cold and variable mountain areas, from the humid rainforest into deserts, from the warm shallow waters into the frigid ocean depths, and from normal warm-water areas into belts of cold upwelling. It is evident that diversity gradients are related to gradients in environmental factors—temperature, humidity,

etc., whether developed on a regional or on a local scale.

On land we speak of the combinations of such physical factors as *weather*, and of the long-period weather patterns as *climate*. These concepts apply equally well to the underwater world, where there are both uniform and seasonal climates, and where, like on land, weather fluctuations may be gradual or catastrophic. Temperatures change, the place of winds is taken by waves and currents, and instead of moisture fluctuations there are changes in salinity (with rather similar physiologic effects). Suspended particles take the place of cloud cover in limiting penetration of solar energy, and excessive sedimentation replaces dust storms and ash falls. We may therefore extend the familiar terms to the world of water, and may speak of *atmospheric weather and climate* on the one hand, and *hydrospheric weather and climate* on the other. Hedgpeth (1957) speaks of *hydrographic* climate in this sense, but to me the adjective *hydrospheric* seems more apt and more consistent.

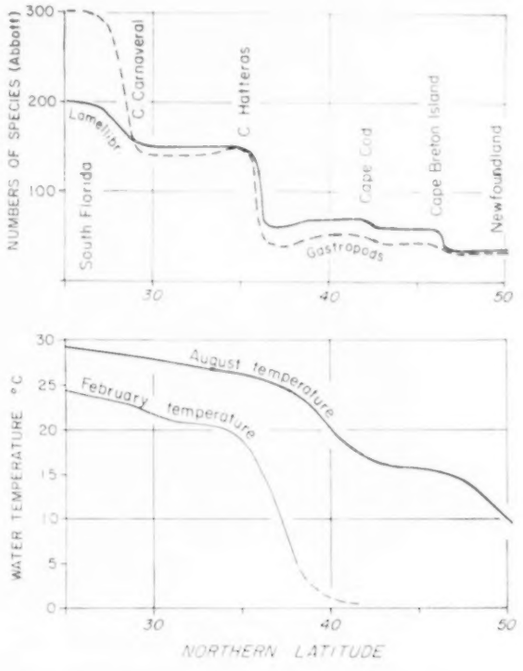

FIG. 10. Comparison of molluscan diversity and marine climate along east coast of Canada and United States. Data from Abbott (1954) and Sverdrup *et al.* (1942).

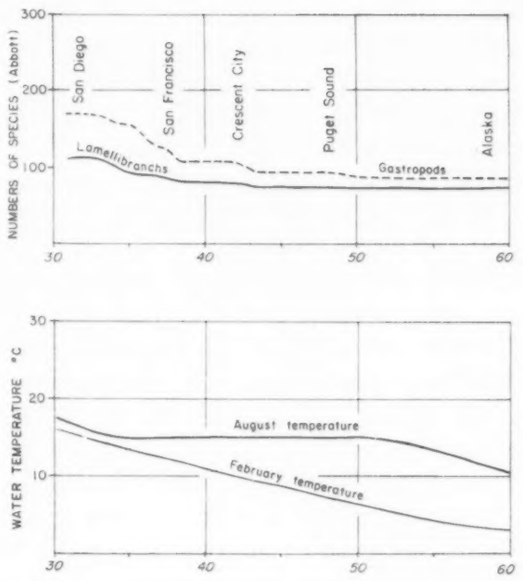

FIG. 11. Comparison of molluscan diversity and marine climate along west coast of Canada and United States. Data from Abbott (1954) and Sverdrup *et al.* (1942).

From a regional standpoint, temperature is the main factor in the climatic control of marine plant and animal distribution. This matter has been considered by Hutchins (1947) and is reviewed by Hedgpeth (1957b). The individuals which compose a population or a species can exist only within certain temperature limits; and within this viability range, there lies a further restricted temperature range outside of which reproduction is impossible.

The diversity gradients which have been been observed lend themselves to the following generalization or rule:

The diversity of biotas, on land and in the sea, is greatest in climates of relatively high and constant temperatures, such as those found over much of the tropics, and decreases progressively into the fluctuating and the cold climates normally associated with the higher latitudes.

MOLLUSCAN FAUNAS ALONG THE COASTS OF NORTH AMERICA

Climatic gradients. The east and west coasts of North America show distinct climatic gradients in respect to tempera-

ture, as illustrated in figures 10–11. Along the east coast this gradient is a very sharp one, for here the climatic differences to be expected as an expression of latitude are reinforced by oceanic circulation, which brings tropical waters northward to Cape Hatteras and arctic waters south to Cape Cod. On the West Coast, the opposite situation prevails; here the gradient is a very gentle one, for the circulation modifies the gradient to be expected from latitudinal differences: the northern part of the coastline is warmed by the Alaska current, while the southern shores are cooled by the upwelling California current.

Data desirable. A study relating the diversity patterns of marine organisms to climatic gradients such as these should ideally be based on data which include:

(1) reasonably detailed and evenly distributed systematic treatment of the chosen group along the entire belt studied, so that monographic highs resulting from patchy collecting or overzealous taxonomic splitting are avoided.

(2) restriction to shallow waters, so as to exclude the deeper forms which are under the influence of climates somewhat different from those expressed by the surface isotherms.

(3) accurate data on the geographic ranges of the species or genera chosen as the units of study.

Data used. The full measure of such information probably is not available for any group of organisms along the North American shoreline; yet, for many groups existing data are probably sufficient for a rough approximation which serves to delineate the basic pattern.

Abbott's *American Seashells* (1954) offers a source for such a compilation on mollusks. This magnificent volume, primarily a guide to shallow-water shells, deals with some 1,500 of the more than 6,000 species of mollusks described to date from North American waters. It provides range data. In emphasizing the shallow-water forms, it largely avoids species belonging to deep-water climates. In excluding the rare forms, it avoids the pitfalls of poorly known ranges and monographic highs. The intense activity of shell collectors in Florida has perhaps introduced some bias, but probably not enough to distort the pattern seriously. Though many ranges given extend on into the Caribbean Island arc and into Mexican waters, Abbott's selection in these outlying regions may not be as representative of the actual fauna as it is off the U. S. and Canadian coasts, and therefore the compilations here made do not extend beyond the tip of Florida and the Coronado Islands. All in all, Abbott's selection within these limits appears to be an adequate index to molluscan diversity, and therefore worth the effort of compilation and presentation.

Results. Figures 10–15 show the diversity gradients in mollusks along the East and West Coast of North America, as compiled from Abbott. Snails and clams are considered separately. Among the snails, the naked forms, pelagic species and pyramidellids were not included. No

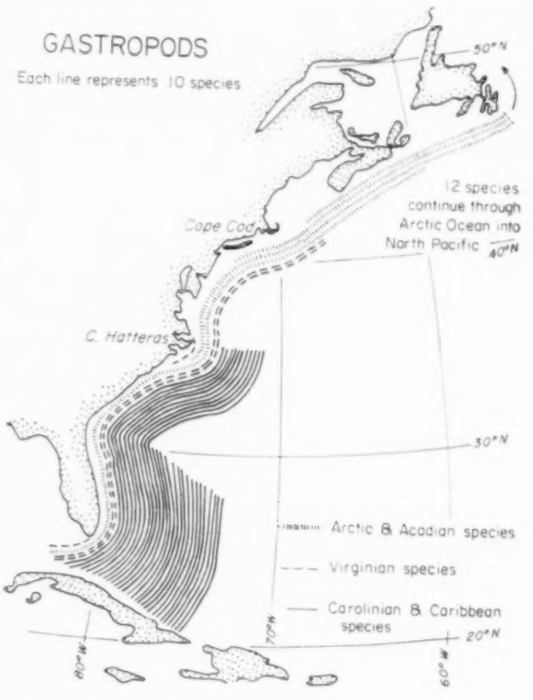

FIG. 12. Diversity gradient of gastropods along eastern coast of United States and Canada. Each line stands for ten species. Data from Abbott (1954).

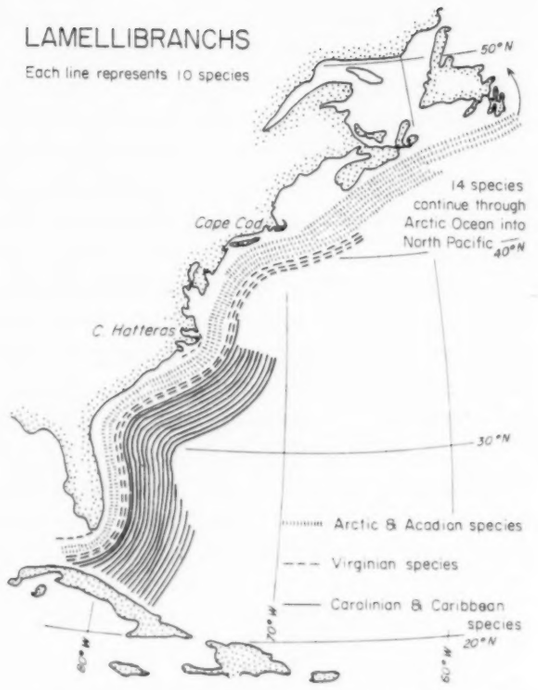

FIG. 13. Diversity gradient of lamellibranchs along eastern coast of United States and Canada. Each line stands for ten species. Data from Abbott (1954).

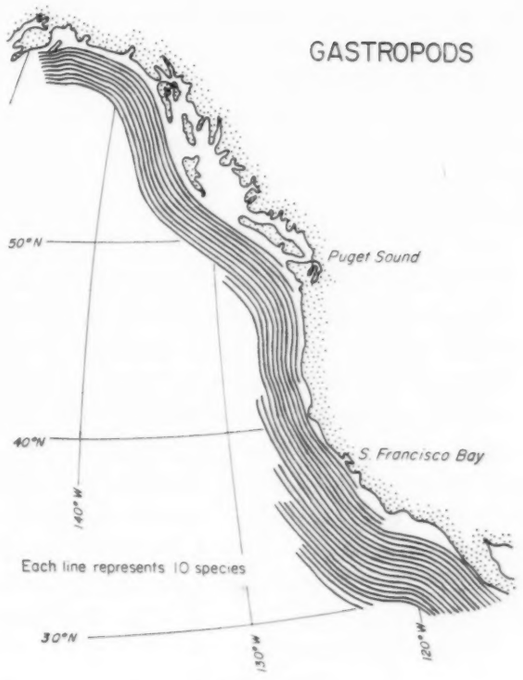

FIG. 14. Diversity gradient in gastropods along west coast of Canada and United States. Each line stands for ten species. Data from Abbott (1954).

compilations were made for the Amphineura, Scaphopoda and Cephalopoda.

The patterns found lend themselves to the following generalizations:

(1) Each case conforms to the rule of correlation between biotic diversity and climate.

(2) The gastropods (mainly epifaunal) illustrate this rule better than the lamellibranchs (which include large numbers of burrowers).

(3) There appears to be no particularly close correlation of the numbers of species with the pattern of seasonal high (August) temperatures, nor with that of seasonally low (February) temperatures, nor with a mean between these: All of these may have some effect, and in addition, the amount of seasonal variation appears to be important, for both graphs show a marked rise in the number of species associated with a convergence of August and February temperatures. No doubt other climatic factors not plotted are of importance; regions strongly influenced

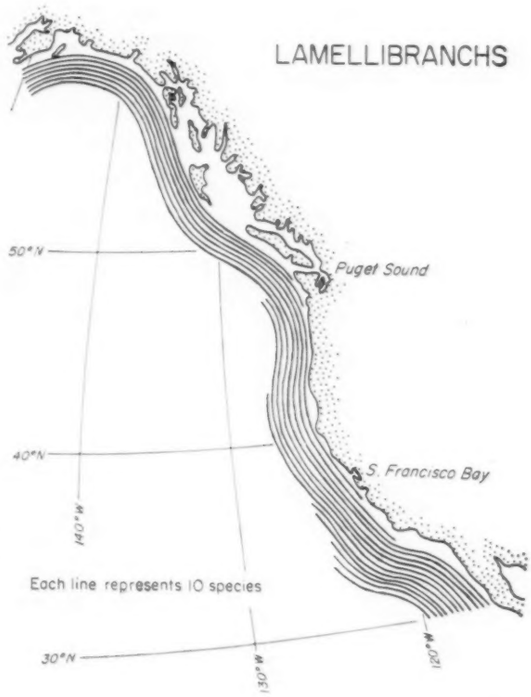

FIG. 15. Diversity gradient in lamellibranchs along west coast of Canada and United States. Each line stands for ten species. Data from Abbott (1954).

by currents are particularly subject to annual or occasional temperature changes of great magnitude and short duration, which would not find expression on normal isotherm maps, and which must have a catastrophic effect upon marine life (see below).

Origin of Diversity Gradients

Biogeographic patterns are the results of two closely interwoven processes: the evolution of organisms, and the evolution of their habitats. Both of these are time-rate problems: Organisms, climates, and the landscape itself are ceaselessly fluctuating, and the extent to which the status quo is altered depends (1) on the direction in which they change, (2) the rate at which they change, and (3) the timespan under consideration.

A model of biotic evolution

Let us consider the change of a fauna by means of a simple model, figure 16. A given fauna, F-1, occupies a certain area at a certain time. It contains 15 species (if you prefer, 150, or 1,500). Some millions of years later the same area is occupied by fauna F-2, numbering 20 species 6 of which are in common with F-1. Figure 16 shows graphically how the change has taken place: Of the original 15 species, 4 were exterminated without having left descendants; 6 survived unchanged; 12 are newly evolved species, of which we may arbitrarily consider 5 to be direct lineal descendants of a similar number of species in F-1, and 7 to be "side-branches"; and 2 immigrated from other regions. It is evident that the difference between F-1 and F-2 depends (1) on the rates at which species are evolved, exterminated, and immigrated, and (2) on the duration of the timespan between F-1 and F-2.

Using this model, we may now inquire into the factors which bring about the addition and subtraction of species, and the ways in which these changes may be influenced by climate. In this inquiry, we shall neglect the immigration factor, to concentrate on (1) ***speciation***, the addition of species to a biota by evolutionary processes, and (2) ***extinction***.

Speciation

Addition of new species (other than immigrants) to a biota is dependent on two factors: (a) the evolutionary potential of existing species, as determined by mutation rates and other factors to be reviewed below, and (b) the rate at which mutants will be selected—something which might be termed environmental receptivity.

Evolutionary potential

The evolutionary potential of any one species would seem to depend on two factors: its genetic variability, and the length of its generations.

Genetic variability involves a number of more or less distinct qualities. One of these is the mutation rate, which may be defined as the ratio of mutant to normal

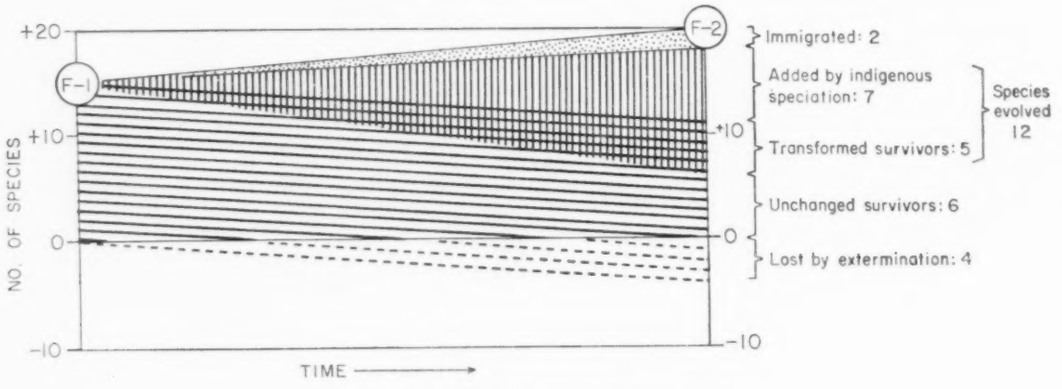

Fig. 16. Model of an evolving biota.

offspring. One might expect this to be related to the complexity of the genetic mechanism—surely the more elaborate the system, the greater the chances for mishaps, alterations and modifications within it—i.e., the greater the chance for mutations. Another is the extent to which mutations are retained and perpetuated within the population gene pool; this factor, also, may be dependent largely upon the complexity of the genetic system. Population size is a third factor: Just as the corner grocery store lacks variety as compared with a supermarket, so very small populations lack genetic variety (and therefore evolutionary potential) as compared with larger ones.

None of these factors appear to bear any relation to climate, and we may therefore incline to discount them as sources of diversity gradients related to climate.

A special aspect of population size, worth a short digression, is the number of offspring produced. Offhand one might think that an organism capable of producing some millions of young per year (such as the oyster) would enjoy a considerable advantage in evolutionary potential over the much less prolific cephalopods, or over certain mammals reproducing at less than one millionth that rate. But this apparent advantage is largely spurious: the production of vast numbers of progeny in oysters is the price paid by a benthonic animal for a defenseless pelagic larval stage, during which the vast majority of the offspring are destroyed in a nonselective fashion. This subject has been discussed by Thorson (1952), who has shown that at the height of the oyster breeding season a single medium-sized *Mytilus* will strain 100,000 oyster larvae in a period of 24 hours. Only a minute fraction of the oyster offspring ever reaches the stage which mutations or gene combinations for a modified filter system or circulatory system can be tested.

Generation length is another factor in evolutionary potential, since the generation is the unit link in the history of any life strain, and new mutants and new gene combinations arise from one to the next. Simpson (1953, pp. 129–132) admits this, but on empirical evidence concludes that the genetic evolutionary potential of organisms is but rarely approached by actual evolutionary sequences. He contrasts the slow evolution of short-generation opossums with the rapid evolution of long-generation elephants, but admits that shortness of generation may be a factor in the rapid evolution of pathogenic bacteria. The development of DDT resistance in certain insects may be another example of rapid evolution facilitated by shortness of generations.

The question which here concerns us is the problem of variations in generation-length with environment: obviously, if species in general mature more rapidly in the tropics than in the higher latitudes, then tropical generations will succeed each other more rapidly; and this might then endow tropical organisms with a somewhat greater evolutionary potential. But observations on maturation rates and generation length show that there is no simple relation between these factors and environment (or latitude). On the one hand, the metabolism of some species seems to vary directly with temperature: Bourlière (1957) points out that the butterfly *Danaus chrysippus* matures in one year in North America and in 23 days in the Philippines, and the beetle *Crioceris asperagi* matures in Germany in one year, whereas *C. subpolita* matures in Java in 25–31 days. Thorson (1952) has reviewed this subject for marine organisms. Some observations on *Ostrea*, *Mytilus*, and *Balanus* show amazingly high maturation rates in the tropics. *Hydroides norvegica* becomes sexually mature 9 days after attachment in Madras, 4 months after attachment in England.

On the other hand, many species and genera appear to be "temperature-adapted," i.e. their tropical representatives and their cold-water forms live at the same rates, and show no significant variation in maturation rate and generation length. Thorson lists observations to this effect

on *Littorina,* decapod crustacea, and echinoids. Furthermore, contrary to widespread opinion, many tropical plants and animals do not reproduce continuously, but have definite breeding periods. Bünning (1956) has pointed out that these may be very long—the sexual reproductive cycle of bamboos, for example, is measured in decades. Nor are the tropical mammals characterized by unusually rapid succession of generations. At this stage, then, it appears that *evolutionary advantage of tropical over high-latitude organisms due to different generation length is at best a minor factor.*

Environmental receptivity

Ecologic niches. A given physical environment provides a variety of possible ways for organisms to make a living, and the organisms themselves greatly multiply the number of these *ecologic niches,* in which properly adapted species can prosper and procreate.

Some workers believe that the main cause for differences in diversity lies in the number of ecological niches available. Thus Bourlière (1957), in writing about animals, states: "It is, however, in the abundance and variety of suitable habitats that one must seek the principle cause of richness of tropical faunas. The environments in which they live is in fact infinitely more diverse than that of temperate latitudes, and many of the habitats of the warm regions have no counterpart in other parts of the world."

The development of major physiographic characters — mountain ranges, alluvial plains etc.—occurs in temperate as well as in tropical regions; certainly there is in the tropics no more diversity of landforms than in other parts of the earth. The related edaphic and microclimatologic features show, if anything, a greater variety within temperate landscapes than within tropical ones: a case in point is the great variety of soil types in the north-temperate regions. Another is the uniform distribution of solar energy in the tropics in contrast to its directional character in the higher latitudes: in the tropics the sun alternately shines from the north and south, as anyone there who tries to grow plants on the "sunny" or the "shady" side of the house will find out in the course of the year. Hence, the striking vegetation contrasts between the north and south flanks of hills, which we tend to take for granted, are not to be found in lower latitudes. Even the change in seasons increases the number of physical niches.

Among the various ecological features of the tropics, it is the organic ones which provide such a rich range of habitats, as discussed by Bünning (1956). Bourlière, in illustrating the richness of tropical environments, cites the variety of animal habitats provided by that most complex of all plant associations, the tropical rainforest. But this accounts neither for the astonishing diversity of tropical plant life, nor for the marine animal diversity gradients which are not associated with such highly organized plant communities.

Differences in selection. Since Darwin's time, the process of selection, resulting from the struggle for survival, has been considered to be one of the main springs of the evolutionary process. And, since biotic diversity is the result of evolution, we may well ask whether differences in the manner of natural selection may partly account for biotas of different diversity.

Schmalhausen (1949) and Dobzhansky, (1950) among others, believe that this is the case.

The size to which a given population may grow depends on its rate of reproduction, and on the pattern according to which individuals are killed off (I use the term pattern rather than rate, for the rate may vary from one stage of life history to another). The nature of the survivors will determine the course of evolution. Two things are therefore of great importance: (1) the selectivity (as opposed to randomness) of killing (or, survival); (2) the direction or directions in which variation is encouraged and accepted by the environment.

Selective factors may be divided arbi-

trarily into two groups: organic and inorganic. Both operate everywhere. At all places, some organisms die because of inclement weather; and others die because of lack of food, or because they are eaten by others, or because they fall prey to disease. But the relative balance between these two sets of factors varies from place to place.

Darwin, Schmalhausen (1949) and Dobzhansky (1950) all call attention to one aspect of this matter: In the high latitudes, one is struck by the havoc wrought among organisms by the physical environment: the changing seasons, and the thereon superimposed catastrophes of weather take a large toll. And, as these investigators have pointed out, the selection accomplished by winter storms, summer droughts etc. is rather crude: mainly such disasters result in random killing of large numbers of individuals. To be sure, organic selective factors are also at work here, since individuals compete with each other, prey and are preyed upon, and succumb to parasites and diseases. And these factors appear to be more selective, in that they involve intense individual competition, and put a premium on individual excellence. But the point is that *much of the killing in high latitudes is done by the less selective inorganic forces.*

In the tropics, on the other hand, the physical environment is more benign to most organisms, and the highly selective interorganic struggle for existence is more apparent. The struggle of plants for light in the rain forest, and the shyness of tropical mammals are dramatic expressions thereof. Dobzhansky believes that this difference may be an important factor in the differential evolution of high-latitude versus tropical biotas.

But the matter is really somewhat more involved than this. The extensive random killing in the high latitudes is largely offset by higher rates of reproduction (Schmalhausen, 1949). And the competition between organisms in the tropics is so apparent because of such a large number of species present. As in the case of the ecologic niches, we are in danger of circular reasoning: is the diversity of tropical biotas due to more intense competition, or is the more intense competition an outcome of the more diverse biota?

One thing appears fairly clear, although it seems not to have been emphasized: In a biota lacking diversity, a great deal of the competition is between members of the same species. This effect is intensified if these species produce vast numbers of progeny, as many of the high-latitude plants and animals do. *In the high latitudes, organic selection results to a great extent from competition of an individual with other members of his species; in the tropics, it tends to be much more a competition of one individual against the members of other species.* This difference must have some effects on the patterns of evolution; to some extent it is a result rather than a cause of differences in diversity, but in part it is linked to the numbers of offspring, and may therefore have had some effect in the differentiation of high-latitude and tropical faunas and floras.

There is, however, a much more direct difference in selection: the tropical temperatures (continental and shallow-water) are nearer the mid-point of the temperature range which protoplasm can endure than are the high-latitude temperatures. Furthermore, the tropical temperatures show less seasonal variation (as has already been mentioned). As a result, tropical conditions permit a wider range of physiologic and structural variation than do high-latitude conditions, which seasonally approach the lower limits at which life can be maintained, and at other times may range into temperatures comparable to those of the tropics. Thus *the tropical environment is receptive to wider latitudes of physiologic variation—to a wider range of mutations—than is the temperate and polar environment.* And this, it seems to me, may be one of the most important factors in the equation which has produced the biogeographic pattern under discussion.

Extinction

In our model (fig. 16) the diversity of F-2 is determined not only by the rate at which species are added, but also by the rate at which they have disappeared. They vanished in two ways: by evolving into new species, and by becoming exterminated. In a way these two modes of disappearance are more alike than their separation on the model would suggest, inasmuch as the transformation of one species into another (a into b) involves the preferred killing-off of individuals of type a. For practical purposes here we may, however, restrict our attention to the outright eradication of species without descendants.

The question is: how does extinction vary with climate; and do old species survive more readily in the tropics than in the higher latitudes?

Extinction of a species can be a sudden process, as demonstrated by the passenger pigeon. On the other hand extinction of a species or a larger taxon can be a very gradual kind of process. This may involve the gradual restriction of members to a smaller and smaller area, where the form may persist (and may be very prominent) for some time as a relict. Or it may come about by the gradual thinning of population density throughout its range, until the spacing between individuals interferes with reproduction.

The mixed and diffuse population patterns of the tropical rainforest and the low density of given species populations suggest that in the tropics extinction may come about chiefly as a result of this gradual dilution. The patchy population patterns and the prevalence of relict population in the higher latitudes suggest that here the relict pattern dominates. In the former case continued organic competition is likely to strike the final blow; relict patches, on the other hand, are particularly vulnerable to the accidents of weather mentioned before. Which extinction mechanism is the more efficient, and what role, if any, such differences have played in the development of diversity gradients, under conditions of constant climate, remains to be seen.

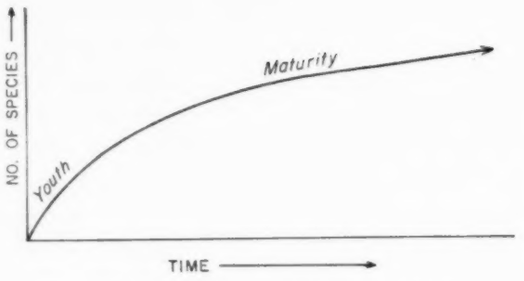

FIG. 17. The curve of biotic maturity.

The time factor and biotic maturity

So far we have considered only relative rates of speciation and extinction as related to climate. We have not and shall not here deal with absolute rates. Neither have we considered (1) relative changes in rates through time, and (2) the evolution of habitats through time.

The maturity model

Again, let us refer to a model, figure 17, simple at the expense of being somewhat remote from reality. We assume a given physical setting without organic inhabitants, and then introduce a limited number of immigrants to start a biota. Most of us would surely agree that in this kind of a setting the availability of many unfilled ecologic niches would lead to a rapid evolution of species and a corresponding diversification of the biota: the diversity curve rises in a steep gradient. To some degree this is a self-generating process: as plants evolve in a first approximation to fill all of the available physical niches, they provide the basis for a first wave of animal evolution. Secondary refinement will lead to the evolution of many species more closely adapted to specific subniches, and these will largely replace their more generalized ancestors; and so the process may go on, almost *ad infinitum*. From the simple dependence of plant on soil, air, sunlight and water, and of animals on plants, there will arise commensalism and symbiosis of

plant-with-plant, animal-with-plant, animal-with-animal, and even 3-way relationships. At this level of complexity we reach the stage shown by tropical coral reefs and rain forests. By now the curve of diversity increase has probably flattened out, as the rate of extinction of old species approaches the rate at which new ones are evolved, but I see no reason why the rise could not continue even beyond the richest biotas existing today.

We may speak of the different parts of this curve as youthful and mature, and accordingly of *degree of biotic maturity.*

From what has gone before, we may conclude that such a curve would rise somewhat faster in the tropics than in the temperate latitudes, and would rise slowest in the polar regions (fig. 18). Yet, the basic patterns should remain the same: evolution within each may be expected to lead from simple biotas to more and more diversified ones, as a result of increasingly specialized adaptation and of more elaborate interrelations with other organisms.

We now face two possibilities: either the biotas of the major climatic belts we have discussed have been evolving for the same length of time, and the differences in their diversity are simply a result of different rates of diversification (slope of the maturity curve) as shown in figure 18. Or the time factor itself is involved as well: some biotas have attained higher diversification (maturity) because they have been evolving steadily over greater periods of time; others are truly immature,

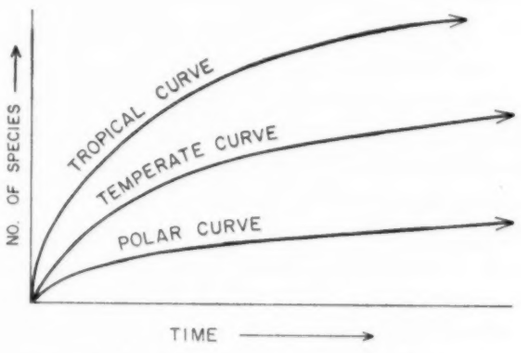

FIG. 18. Comparison of tropical and other maturity curves.

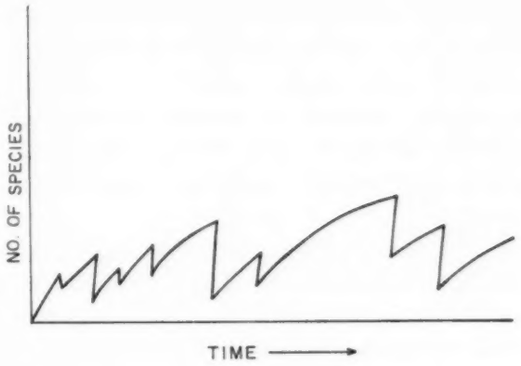

FIG. 19. History of organic diversity in an area subjected to climatic fluctuations during geologic time.

having originated much later or having been set back by periodic decimations (fig. 19). So long as we know so little of the quantitative aspects of evolution, the biotic patterns alone are not likely to give us a definitive answer to this question.

Climates through geologic time

If there is any one theme which runs through the geological record, it is that of ceaseless change.

Given a spheroidal globe, rotating on an inclined axis and circling the sun in a certain orbit, we may be sure that the solar energy received in the equatorial regions was always markedly greater than that received at the poles, and that the reception of this energy at the equator varied but little through the year, whereas it was subjected to seasonal variations in the polar areas. But beyond these basic invariables there lies a host of variable factors. Some of these are known to have operated, others are at the present stage of knowledge no more than possibilities and probabilities.

The variation is in the rate and pattern of redistribution of this energy. The rising of air in the tropical belt, its descent at the horse latitudes and in the polar regions, and the turbulent mixing in between greatly affect weather and climate —as does to a lesser extent the circulation of water in the oceans. Both are to a very large extent governed by the distribution of land and sea. This distribution is less

important in the tropical belt, which has probably always extended over a mixture of land and sea in which the sea was dominant, than it is in the small polar regions, which have at times been dominantly continental, at others dominantly marine.

Land areas have their surface layer heated quickly to comparatively high temperatures by incident sunlight. Downward conduction of heat is slow, but loss to the atmosphere is rapid, and thus little of the energy becomes stored. As a result we experience the extremes of "continental climates." In water covered areas, the solar energy is only partly absorbed at the surface, and heats deeper layers as well. Partly because of this, partly because of the much greater specific heat of water, and partly because of convection, the water mass stores the solar energy at lower temperatures and in a form which is gradually released to the atmosphere. As a result we speak of the ameliorating climatic effects of water bodies. In oceanic areas water circulation itself introduces an additional ameliorating factor, which tends to keep the circum-polar oceans warmer in winter than the circum-polar landmasses.

Four polar models. To visualize possible climatic extremes we may consider four situations; (1) a pole in the midst of an oceanic area; (2) a pole in the midst of a large continental area; (3) a pole in a limited continental area surrounded by seas (the present South Pole); and (4) a pole in a sea surrounded by continental areas (the present North Pole).

In the first model, the polar regions would be of moderate temperature in summer and in winter, maintained during the latter from the vast thermal reservoir of the oceans. A polar climate with extensive ice, such as we know today, would not exist. Neither would there be the giant struggle of the polar and the equatorial air masses, which stamps the present "temperate" region with their catastrophic character. Instead, the temperate regions around this polar ocean area might show climates like those of present-day New Zealand, Tasmania, and coastal Alaska.

Model 2 would provide the opposite extreme. The polar landmass would be subject to extensive heating in the summer and to extremely frigid winter weather. Great polar air masses would make extensive inroads into the subtropical fringe during the winter. The "temperate" areas would thus be even less temperate than those we know. Ice sheets might accumulate in the central area, but the summer heat and lack of moisture would make widespread glaciation improbable.

Models 3 and 4 are available for study. In model 3, the polar landmass is utterly frigid, but the surrounding oceanic belt (famous for its violent storms) takes the brunt of the atmospheric mixing, and beyond them lies a truly temperate zone, protected from the catastrophic inroads of polar air masses.

In model 4, the polar ocean helps to ameliorate the climatic extremes of the polar area and adjacent regions, but provides a source of moisture which encourages the development of glaciers, and may thus cause a widespread development of what we normally think of as polar conditions. The history of the northern hemisphere during the last million years bears witness to this.

The history of the polar regions. Present polar and near-polar continental areas have in the past been widely covered by seas, just as have other continental regions. The existence of these seas is recorded in the form of marine sedimentary rocks. Thus we need no further proof that extensive changes of land and sea have taken place in the polar regions, and that profound climatic effects must have been felt in the polar and temperate regions.

But, beyond this, different lines of evidence suggest strongly that the poles and the earth's crust have shifted with respect to each other. The fossil record shows biotic patterns difficult to reconcile with the present geographic grid: for example, the lower Paleozoic faunas of South

America are very limited in variety (immature?), while exceedingly rich faunas and coral reefs thrived from central North America to the Arctic and to Scandinavia. The patterns of ancient glaciations, while still far from well understood, are also difficult to reconcile with the present pole positions. Remanent magnetism in sediments and lava flows offers a possible clue to pole positions of the past, and work to date suggests extensive polar shifting.

Such polar wandering might have come about either by shifting of the earth's crust over the underlying "mantle," or by displacement of the globe as a whole relative to its axis of rotation, or by extensive displacements of oceans and continents relative to each other and to the earth's axis. The work on remanent magnetism and the current discoveries of extensive wrench faulting in the earth's crust offer support for such theories.

Conclusions. It is concluded from this that widespread climatic changes have been a normal feature of earth evolution. The broad tropical belt, with its mixture of lands and seas, has probably been the most constant feature of the earth, although its position may have shifted. Polar climates of the present type have probably existed at some times and not at others. The temperate zones have undergone severe changes involving great expansions and contractions (as during the Pleistocene), changes from truly temperate to rigorous and subject to seasonal catastrophes, and possibly extensive shifting over the globe.

From these considerations it appears probable that the tropical marine and continental biotas of today are the products of a long and relatively undisturbed evolutionary history, and are truly mature, while the polar and temperate biotas have experienced a turbulent history of mass extinctions and gradual re-evolution. At times they have been more mature than at present. The great Tertiary mammalian faunas of North America, and the rich marine fauna of the East Coast Miocene, may be examples of comparatively mature faunas adjusted to a truly temperate climate in the temperate zone. They were decimated by the more rigorous and catastrophic climates which may have had their beginnings in the Pliocene and reached their climax in the Pleistocene glacial stages. Our present North American temperate biotas probably represent a mixture of polar types and the most hardy survivors of the pre-Pleistocene temperate biotas, undergoing rapid evolution in a comparatively immature stage of the curve (fig. 17). This evolutionary surge is not likely to be carried very far: So long as the North Pole remains in the Arctic Ocean, a kind of self-induced oscillation involving freezing and thawing of the arctic seas, waxing and waning of ice caps, and rise and fall of sea level (Ewing and Donn 1956, 1958) may continue to bring alternate glacial advances and retreats, and may effectively prevent the northern temperate biotas from becoming mature.

Local diversity gradients

We may now return to glance at the local climatic diversity gradients, only mentioned above. The tropical rain forest and the warm tropical sea are normal and enduring features of the tropical belt. Mountain ranges, rainshadow deserts and areas of cold upwelling are geologically transient phenomena; their biotas are either lately evolved in place, or have been subjected to drastic migrations in the not-too-distant geologic past. They are therefore immature as compared to the normal tropical biotas.

General Conclusions

The diversity of biotas, on land and in the sea, is greatest in climates of relatively high and constant temperatures, such as those found over much of the tropics, and decreases progressively into the fluctuating and the cold climates associated with the higher latitudes. It is proposed that this pattern results largely from the following causes:

(1) Biotas in the warm, humid tropics are likely to evolve and diversify more rapidly than those in the higher latitudes, mainly because of a more constant (favorable) normal environment, and relative freedom from climatic disasters.

(2) Biotic diversity is a product of evolution, and is therefore dependent upon the length of time through which a given biota has developed in an uninterrupted fashion. The low-level tropics represent that part of the globe which has been least affected by climatic fluctuations. Such factors as marine transgressions and regressions in the polar regions, possible polar wandering, and oscillations of glacial polar conditions have caused extensive fluctuations in polar and "temperate" climates, and have thereby profoundly disturbed the normal course of evolutionary diversification. The tropical coral reef and rainforest biotas are considered as examples of mature biotic evolution, whereas the biotas of the regions covered by Pleistocene ice sheets are prime examples of "immature" relicts of the more mature temperate Tertiary faunas and floras. We thus return to A. R. Wallace's words (1878): "The equatorial zone, in short, exhibits to us the result of a comparatively continuous and unchecked development of organic forms; while in the temperate regions there have been a series of periodical checks and extinctions of a more or less disastrous nature, necessitating the commencement of the work of development in certain lines over and over again. In the one, evolution has had a fair chance; in the other, it has had countless difficulties thrown in its way. The equatorial regions are then, as regards their past and present life history, a more ancient world than that represented by the temperate zones, a world in which the laws which have governed the progressive development of life have operated with comparatively little check for countless ages, and have resulted in those wonderful eccentricities of structure, of function, and of instinct—that rich variety of colour, and that nicely balanced harmony of relations which delight and astonish us in the animal productions of all tropical countries."

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STAGE OF SPECIATION OF TWO ALLOPATRIC POPULATIONS OF CHORUS FROGS (*PSEUDACRIS*)[1]

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The best estimate of the course of geographic speciation comes from analysis of disjunct, allopatric populations in respect to their differentiation in attributes that might function as isolating mechanisms, and in morphological characters. Various east-west disjunct populations of vertebrates on the Gulf coastal plain of the southern United States, with presumed Pleistocene origins (Blair, 1958b), afford excellent material for such an analysis. The present report deals with two geographically separate populations of chorus frogs. One, *Pseudacris ornata,* occurs on the coastal plain of the southeastern United States and has its western distribution limited at the Mississippi Embayment. The other, *P. streckeri,* occurs in the broad forest—grassland ecotone just west of the deciduous forest in Texas and Oklahoma and has its eastern distribution limited by the forest. Relict populations of *P. streckeri* occur in Illinois and Missouri.

For these two allopatric species we have obtained a measure of the morphological differentiation, of the differentiation in mating call and certain other attributes that might affect interbreeding if the populations should become sympatric, and of the discrimination by ripe females of one of the species when exposed to the calls of males of both.

Materials and Methods

The morphological comparisons are based on museum specimens of 91 male and seven female *Pseudacris ornata* from northern Florida and southern Georgia, and 483 male and 52 female *P. streckeri* from Texas and Oklahoma. All specimens of the latter species are in the Texas Natural History Collection at The University of Texas.

The comparisons of mating calls are based on tape recording of 10 individuals of *ornata* and 26 of *streckeri.* All these recordings except those of three *ornata* borrowed from Cornell University are on file in the bioacoustical laboratory at The University of Texas. Analyses of the calls have been made by use of a sound spectrograph (Sona-Graph) which portrays frequency, intensity, and time.

The female *streckeri* used in the discrimination experiments were obtained in central Texas. The simple apparatus used in the experiments consists of an enclosure with a speaker at each end so that choices of recorded calls can be presented to a female. This apparatus is modeled after that described by Martof and Thompson (1958) and has been described in detail by Littlejohn and Michaud (1959). The recorded calls are played through two 2.5 inch diameter loudspeakers, one at each end of the tank, using repeating loops of tape. The work is done in a temperature-controlled room at 19.5 ± 2.0° C.

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Paul Kellogg kindly made available recordings of the calls of *P. ornata* in the Cornell University Library of Natural Sounds. The sound spectrographic work was done by Wayne H. McAlister and M. J. Fouquette, and the latter made the morphological measurements and did the statistical analyses.

Morphological Differentiation

Frogs of the two allopatric populations are rather similar, but there are subtle and subjectively describable differences as well as differences in size and proportions. The color pattern is variable in both, but *streckeri* tends more to spot-like bars of dark pigment on the dorsum, while *ornata* tends more to have two dark longitudinal dorsal bars. The dark bars or spots may be greatly reduced in individuals of either species. The ventral surface appears more granular in *streckeri* than in *ornata*. In general terms, *ornata* is more slender, with more pointed snout and longer and more slender arms and legs; *streckeri* is short, fat, squatty, with shorter, broader snout and short, broad arms and legs (Wright and Wright, 1949). Comparison of the measurements of the available specimens (table 1) provides support for the statement of these general differences between the two species. The larger size of *streckeri* is reflected in the significantly greater snout-urostyle length, although the greater robustness of this frog cannot be shown by this linear measurement. The relatively longer head of *ornata* is indicated by the ratio of head length to head width. The head is significantly longer in male *streckeri* in relation to snout-urostyle length than in male *ornata*. There is no significant difference in this respect between the females, but the sample of female *ornata* is inadequate. Both the tibia and foot (including toes) are significantly longer in relation to snout-urostyle length in *ornata* than in *streckeri*.

The adaptive significance of the obvious morphological differences between the two populations of frogs can only be hypothesized at present. Circumstantial evidence suggests that the differences in size and proportions are probably adaptive to the rather different environments of these two frogs. The larger, more robust body of *streckeri*, with less surface area in relation to mass, would presumably have a selective advantage in terms of water conservation in the more xeric western distribution area of this frog. At least two other complexes of anurans with eastern and western differentiates (*Acris gryllus—A. crepitans; Bufo woodhousei fowleri* and *B. w. woodhousei*) show larger body size in the western member and smaller in the eastern one. The situation of longer legs (and feet) in the eastern, low-coastal-plain population and shorter ones in the more western population is paralleled in *Acris*. The significance of this is obscure, although it could be a surface to mass phenomenon related to water conservation.

Potential Isolating Mechanisms

Time and place of breeding

These are both winter-breeding frogs that spawn in temporary pools after winter

Table 1. *Comparisons of external morphological measurements of* Pseudacris streckeri *and* P. ornata

Characters	Males Means and standard errors		Females Means and standard errors	
	streckeri (N = 483)	*ornata* (N = 91)	*streckeri* (N = 52)	*ornata* (N = 7)
Snout-urostyle length	31.93 ± 0.11	29.36 ± 0.14	33.87 ± 0.17	30.97 ± 0.25
Head length/head width	0.876 ± 0.002	0.940 ± 0.005	0.873 ± 0.006	0.931 ± 0.017
Head length/snout-urostyle	0.338 ± 0.001	0.330 ± 0.001	0.336 ± 0.001	0.328 ± 0.004
Tibia/snout-urostyle	0.433 ± 0.001	0.457 ± 0.002	0.426 ± 0.003	0.449 ± 0.006
Foot/snout-urostyle	0.707 ± 0.002	0.778 ± 0.003	0.688 ± 0.005	0.775 ± 0.012

TABLE 2. *Measurements of mating calls of* Pseudacris ornata *and* P. streckeri

Species and locality	N	Air temp. °C.	H_2O temp. °C.	Mean dominant freq. (cps)	Mean duration (sec)	Mean interval (sec)
P. ornata—Newton, Ga.	7	18.0	16.5	2750	.04	.34
P. ornata (Cornell record)	3	—	—	2830	.04	.40
P. streckeri—Austin, Tex.	1	22.0	19.0	2350	.06	.24
P. streckeri—Austin, Tex.	17	21.5	18.0	2300	.05	.35
P. streckeri—Luling, Tex.	3	19.0	21.3	2225	.04	.35
P. streckeri—Brenham, Tex.	5	23.0	22.0	2430	.04	.21

rains. The breeding season of a *streckeri* population at Austin, Texas, has been carefully observed over a period of four years. The beginning of the breeding season, as evidenced by calling males, varied from October 22 to January 23. These variations were influenced largely by rainfall and to a lesser degree by temperature. The end of the breeding season varied from March 2 to April 29. The breeding season of *ornata* in Florida has been stated (Carr, 1940) as extending from November 3 to March 2. The close approximation of their breeding seasons and their habits of breeding in temporary pools indicate that if these two populations became sympatric there would be no initial restriction on interbreeding through habits of breeding at different times or places.

Genetic incompatibility

Mecham (1957) obtained normal-appearing, viable F_1 's in the cross of ♀ *streckeri* × ♂ *ornata*. One hybrid male, in an attempted backcross to a *streckeri* female, fertilized no eggs and appeared to be producing no viable sperm. Mecham (1959) made the reciprocal cross and obtained only sterile males.

It would appear, therefore, that these populations in isolation have differentiated sufficiently that there is a sterility barrier to their interbreeding, although there is no proof at present that the barrier is complete.

Mechanical incompatibility

The difference in robustness and body size must be considered as a possible isolating mechanism. However, the difference is less than that between *Bufo woodhousei woodhousei* and *B. w. fowleri* which apparently interbreed freely in a narrow zone of secondary contact (Meacham, 1958).

Mating call

The call in both of these species is a short, finely tuned whistle that derives most of its energy from a dominant frequency band between 2,000 and 3,000 cps and from the first higher harmonic of this band at 4,000 to 6,000 cps. This call is repeated in sequence, with a silent interval between calls of about one-third of a second. Comparison of the available sound spectrograms of calls (table 2) shows that there is no significant difference between the two species in duration of the call or in interval between calls (measured as the silent period). The only apparent difference in call is in the frequency. The dominant frequency band rises about 200 cps from beginning to end, and for purposes of comparison the dominant frequency has been measured at approximately the middle of the call. As may be seen from table 2, the dominant frequency averages about 500 cps higher in *ornata* than in *streckeri* and twice that in the harmonic. The range of variation in the two species barely overlaps, as one of the 10 *ornata* and one of the *streckeri* had a dominant frequency of 2,500 cps.

Call discrimination

Discrimination experiments were run, by methods referred to earlier, in order to test whether the rather slight differences in mating call might serve as species

TABLE 3. *Details on calls used in discrimination experiments*

Species and locality	Recording temperatures, °C.		Duration (seconds)	Dominant frequency (cps)
	Air	Water		
P. streckeri				
6 miles S.S.E. of Luling, Texas	19.0	21.3	0.04	2280
P. ornata				
Newton, Georgia	18.0	16.5	0.03	2640

identification cues for the females in going to the calls of the males. Gravid *streckeri* females, collected in amplexus, were released in the test tank, and their orientation toward the loudspeakers from which the calls of *ornata* and *streckeri* were being played was noted. The particular calls between which the females were given a choice differed in that the call of the *ornata* had dominant frequency of 360 cps higher and a duration 0.01 seconds shorter than that of the *streckeri* (table 3).

In the test runs, contacts with a loudspeaker were scored if a female fulfilled any one of the following conditions:

1. circled the speaker at a distance of 4–10 inches;
2. jumped at the speaker, making a transient contact with it, then passing on for about 6–10 inches;
3. sat about 2 inches away from the speaker;
4. made deliberate contact with the speaker by jumping or climbing over it or sitting on or against it.

Only one contact was scored for a sequence involving orientation which could include two or more of the above conditions. However, if a frog stopped circling and moved away, then returned again, or if a frog moved more than 6 inches away after fulfilling conditions 2–4, and returned again, then subsequent contacts were also scored.

Frogs were initially released at the center of the tank, either two or three being tested at the same time. After the frogs had contacted a speaker, connections were switched so that the calls now came from opposite ends. Such exchanges of calls were sometimes made several times during the testing of a particular frog and at least once for each individual. The interval between changes is referred to as a trial (Littlejohn and Michaud, 1959).

The results given in table 4 are from 29 frog-trials involving a total of nine female *streckeri*, eight from Travis County, and one from Lee County, Texas. As can be seen, 35 contacts were scored with the *streckeri* speaker and four with the *ornata* speaker. Two of the latter occurred directly after release and could have resulted from random movement; three were made by the same frog, and all occurred during one test run involving three frogs.

A reactive frog moved decisively and accurately toward the sound source of the *streckeri* call and ultimately contacted the speaker. On several occasions females after contacting the *streckeri* speaker moved to within about a foot of the *ornata* speaker, paused, faced it for a few seconds, then turned and swam back to the *streckeri* speaker, usually to make another contact. On three occasions females clasped other females in front of the

TABLE 4. *Summary of contacts made with loudspeakers by 9* P. streckeri *females.* See text for explanation. ? refers only to the category into which the contact should be placed.

Loudspeaker	Class of contact				
	1.	2.	3.	4.	Total
P. streckeri call	1	1	13+?1	19	35
P. ornata call	0	1	?1	2	4

$Chi^2 = 24.6$, P less than 0.001.

streckeri speaker. These lasted from about 15 seconds up to three minutes. Times taken for females to move from the release point at the center of the tank to contract the *streckeri* speaker some 42 inches away ranged from 3.17 to 5.50 minutes (mean = 4.78), and for the total distance between the speakers of about seven feet from 0.25 to 8.50 minutes (mean = 2.54).

On one occasion two females were offered only the calls of *ornata* through one of the speakers over a six minute period. During this time they came within two inches of the speaker five times but were never actually observed to make direct physical contact (types 2 and 4) with it. When *streckeri* calls were started at the opposite end, the two frogs immediately left the vicinity of the *ornata* speaker and moved directly to the *streckeri* speaker, contacting it by persistently jumping against it. They then assumed a position of amplexus. All this activity occupied about one minute from the starting of the *streckeri* calls.

The results of these tests indicate decisively that the female *streckeri* are attracted to the calls of male *streckeri* but not to the slightly different calls of the male *ornata*. The females generally respond to the calls of the male of their own species by making physical contact (with the loudspeaker in the experimental situation), which in these frogs normally elicits clasping by the male. Blair (1958a) regarded the relatively slight difference in call of these two species as being insignificant as an isolating mechanism. The results of the present experiments indicate that this opinion was unjustified.

Discussion

This pair of disjunct, allopatric species provides an excellent opportunity for assessing the process of geographic speciation. The time element must remain uncertain in the absence of a fossil record, but the fragmentation of the ancestral range is reasonably attributable to the effects of Pleistocene climatic change (Blair, 1958b). The disjunction could have come as recently as the beginning of the Wisconsin, but it cannot be proved that it did not come earlier.

Differentiation in the various attributes discussed above presumably came after geographic isolation of the two populations, but there is no way of knowing how much intraspecific geographic variation in morphological and ecological characters existed prior to the time of the disjunction. Nevertheless, the two populations are now sufficiently different in morphology that they have been regarded as distinct species by taxonomists. The most notable differentiation (in body size and robustness) seems probably to have come from differential selection in different moisture environments. The two populations have differentiated genetically to the extent that genetic incompatibility, expressed in sex-ratio disturbance and sterility of the F_1 would certainly limit and possibly block gene transfer between the populations if they should ever become sympatric.

The two populations have remained essentially undifferentiated in respect to breeding season (winter) and the kinds of water (temporary rainpools) in which spawning occurs. The mating call, with a primary function in each population of aggregating the sexes for reproduction, has differentiated slightly, and apparently as a byproduct of the adaptive differentiation in body size and robustness. As a general rule, the dominant frequency varies inversely with body size in anurans (Blair, 1958a). The relatively slight differentiation in mating call, which we have shown, however, to be adequate for species discrimination, and which apparently came about incidental to adaptive differentiation of the two populations, exists as a potential isolating mechanism of great significance if the populations should ever establish contact.

We construct the speciational history of these frogs, then, as being initiated by geographic separation, followed by adaptive differentiation that led indirectly to

the acquisition of an important pre-mating isolating mechanism (call difference) and possibly along with genetic drift, to the acquisition of an important post-isolating mechanism (genetic incompatibility). This case history closely parallels the one reported for the *Peromyscus maniculatus—P. polionotus* complex (Blair and Howard, 1944) where there is strongly adaptive differentiation in color pattern, interspecific discrimination, and partial genetic incompatibility between disjunct, allopatric species of presumed Pleistocene origin. Both of these cases fit the often termed "Mullerian" model of geographic speciation (Muller, 1940).

In closing, however, we would like to emphasize that these cases represent only one of the possible pathways of geographic speciation and should not be the basis for any broad generalization. Pre-mating isolating mechanisms may evolve without concomitant genetic incompatibility; populations may establish sympatry and undergo reinforcement of less completely effective isolating mechanisms than the ones under discussion here would appear to be (Blair, 1958a).

Summary

The allopatric chorus frogs (*Pseudacris ornata* and *P. streckeri*) of the southern United States are analyzed in respect to their morphology, breeding habits, genetic compatibility, and mating call. The populations have presumably been separate since some time in the Pleistocene. The most noticeable morphological differentiation is in body size and proportions, with the larger size of *streckeri* being possibly adaptive to a more xeric environment than that of the smaller *ornata*. The two populations have not differentiated in time of breeding or in the kinds of waters (temporary pools) used for spawning. The two species on available evidence show genetic incompatibility of a degree that would restrict and possibly prevent gene exchange if they should become sympatric. The mating call is moderately differentiated in frequency (about 500 cps in the dominant and 1,000 cps in its first harmonic), but the differentiation is sufficient for female *streckeri*, at least, to discriminate between calls of their own species and those of *ornata*.

These two allopatric populations have differentiated sufficiently, presumably while geographically isolated, that they should behave as separate species if they should become sympatric. Differentiation in mating call, which appears to be the most significant attainment of these populations in respect to their evolution into separate species, is possibly a byproduct of their adaptive differentiation in body size.

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A CYTOGENETIC STUDY OF *CLARKIA EXILIS*

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Evolution in the genus *Clarkia* (Onagraceae) has often been accompanied by extensive repatterning of chromosomes, mostly in the form of reciprocal translocations (Lewis, 1953). Thus, *Clarkia* populations may be homozygous for particular chromosome arrangements or they may be structurally heterozygous for one to several reciprocal translocations (Lewis, 1953, 1951; Lewis and Raven, 1958; Lewis and Roberts, 1956; Mooring, 1958; Roberts and Lewis, 1955). In an earlier paper (Vasek, 1958), the author reported that the sterile hybrid between *Clarkia unguiculata* and *C. exilis* was heterozygous for at least three, and probably four, reciprocal translocations and that populations of *C. exilis* may differ by three translocations. That report, however, was based mostly on one interspecific hybrid and on hybrids involving only three populations of *C. exilis*. The present study extends investigation to seven colonies of *C. exilis*, and to three colonies of *C. unguiculata*.

The first objective of this paper is to determine the pattern and extent of translocations within *C. exilis*, and to compare that pattern with the pattern found by Mooring (1958) in *C. unguiculata*. A second objective is to ascertain by analysis of crossability, interfertility and translocation patterns, whether the seven colonies of *C. exilis* are equally distinct from *C. unguiculata*. A final objective is to determine, if possible, the genetic and geographic origin of *C. exilis*.

Clarkia exilis provides desirable material for the type of study contemplated for it has a restricted range of distribution (Vasek, 1958). Thus, the seven colonies investigated sample essentially the entire range of the species. The colonies of *C. exilis* are distributed along the Sierra Nevada foothills in Kern and Tulare Counties, California (see Vasek, 1958, figure 1). Abbreviations used to identify colonies are listed in table 1.

The three colonies of *C. unguiculata* used in this study were selected for various reasons. The colony from Springville (US) is just north of the northernmost colony of *C. exilis* and has some morphological features suggestive of *C. exilis* (Vasek, 1958). Thus, it may have significance in indicating the origin of *C. exilis*. The Kern Canyon (UK) colony is sympatric with the Kern Canyon colony of *C. exilis* and was used in producing the interspecific hybrid reported in 1958. Plants from a colony in Riverside (UR) were included in the present program because they were readily obtainable.

Attempts were made to intercross the 10 colonies in 82 of the 90 possible combinations. The parent plants used in this hybridization program were members of greenhouse populations grown from wild seed. A green house population, representing a colony of *C. exilis*, consisted of a progeny or two of siblings totaling 50 or more plants. An important reason for establishing greenhouse populations on this basis is that meiotic stages in successive buds are farther apart in *C. exilis* than in *C. unguiculata*. Hence, some bud samples of *C. exilis* are likely not to contain cells in first metaphase. If a particular plant had been used as a parent and had not been successfully analyzed cytologically, its chromosome arrangement might reasonably be inferred from analysis of its siblings. With 50 or more bud samples, the probability of 20 or 30 successful analyses is comfortably high. In *C. unguiculata* on the other hand,

TABLE 1. *Abbreviations used to designate colonies of* C. exilis *and* C. unguiculata. The first letter indicates the species and the second letter indicates a geographical locality (listed in order from south to north).

C. exilis

EB	Breckinridge Mountain
EK	Kern Canyon
ER	Rancheria Road
EG	Granite Station
EW	White River
ED	Deer Creek
ET	Tule River

C. unguiculata

UR	Riverside
UK	Kern Canyon
US	Springville

almost all bud samples can be successfully analyzed and hence smaller populations will suffice.

A second reason for having large populations is that 50 plants is a reasonable sample with which to estimate the genetic variation among siblings. The same populations were used as the basis for estimating the morphological differences between colonies (Vasek, 1958).

Hybrid fertility was estimated from the maximum number of seeds per capsule following self pollination and the percentage of viable pollen grains. Pollen grains that stained with cotton blue in lactophenol were considered viable. Pollen grains that did not stain were shrunken and shriveled and considered inviable.

Bud material for cytological study was fixed in 1 : 3 acetic acid-absolute ethanol and stored in 70% ethanol. Cytological observations were made at first meiotic metaphase after squashing pollen mother cells in acetocarmine following hydrolysis in 1N Hydrochloric acid. Chromosome staining, which was often poor, even under phase contrast, was satisfactorily improved by mordanting buds in acetorcarmine for several hours before squashing. No particular effort was made to analyze a specific number of cells per plant but rather analysis continued until a maximum configuration was consistently observed or until there were no more cells.

RESULTS

Crossability

The seven colonies of *Clarkia exilis* are readily intercrossed in all combinations. The three colonies of *C. unguiculata* are likewise readily intercrossed. Numerous intra-specific hybrids are easily obtained indicating that, within either species, there are no particular barriers to fertilization or seed germination.

Interspecific hybrids, on the other hand, are difficult to obtain. Low seed set following interspecific pollinations and poor germination of hybrid seeds suggest genetic barriers to both fertilization and germination. In *C. exilis* × *C. unguiculata* seed set was reduced to a maximum of 10 to 16 seeds per capsule (each species normally produces more than 60 seeds per capsule) and none of the hybrid seeds germinated. In UK or UR × *C. exilis* only a few seeds were produced in some capsules and only an occasional hybrid seed germinated. However, in US × *C. exilis* up to 39 seeds were produced per capsule and 17% of the hybrid seeds germinated. Thus greater success was achieved in crossing *C. exilis* with US than with UK or UR but only when *C. exilis* was used as the pollen parent.

Hybrid fertility

A few plants, derived from selfing plants of the parent populations, were grown, as a control, synchronously with the hybrids. Fertility was about the same as for the year before but sometimes seed sets were slightly higher or lower.

Hybrid fertility, based on pollen stainability, is shown in table 2 and hybrid fertility, based on seed set, is shown in table 3. Most of the hybrid combinations within *C. exilis* have over 90% viable pollen and set over 80 seeds per capsule. Those plants that have lower pollen fertility have higher seed sets and vice versa. Two exceptions are EB × ET and EW × ET which are slightly lower than the figures mentioned, but then their reciprocals ET × EB and ET × EW have

TABLE 2. *Maximum per cent of stainable pollen in the indicated hybrids*

♀ parent \ ♂ parent	EB	EK	ER	EG	EW	ED	ET	UK	UR	US
EB	—	91	91	60	93	91	83	—	a	a
EK	92	—	100	97	99	98	82	—	a	a
ER	88	100	—	98	100	99	86	—	—	a
EG	67	98	98	—	99	99	98	a	a	a
EW	96	99	98	98	—	99	88	—	—	a
ED	93	100	99	99	99	—	88	a	a	a
ET	75	89	89	88	93	82	—	a	a	a
UK	—	b	a	a	a	a	5	—	100	9
UR	a	a	a	a	a	a	3	99	—	—
US	7	6	6	5	7	5	4	6	6	—

a—pollination attempted, but no hybrid obtained.
b—see Vasek, 1958.

high seed sets or high pollen fertility. Within *C. exilis,* then, all hybrid combinations are highly fertile and the level of fertility is as high as, or at least not significantly lower than, the parental populations.

The hybrids between UK and UR are highly interfertile and can be taken, for the present, as a standard for *C. unguiculata.* Surprisingly, US hybrids are about as fertile in combinations with *C. exilis* as in combinations with *C. unguiculata* but this level of fertility is quite low. In both types of hybrids 9% or less of the pollen is stainable and 13 or fewer seeds per capsule are set. On the basis of hybrid fertility, then, US represents a genetic system separate from both *C. unguiculata* and *C. exilis.*

Cytology

Plants of the parental greenhouse populations were studied cytologically to determine whether they were structurally homozygous. Within *C. exilis,* 4886 metaphase cells from 185 plants of seven populations were successfully analyzed. Three (out of 39) plants from Tule River and six (out of 30) plants from Deer Creek had a ring of four chromosomes indicating that those plants were heterozygous for one reciprocal translocation. All the plants from the other five colonies were structural homozygotes except that one cell in each of two plants from Breckinridge Mountain (398 cells examined) showed a ring of four chromosomes. The extremely low frequency of structurally

TABLE 3. *Maximum number of seeds set per capsule after self-pollination of the indicated hybrids*

♀ parent \ ♂ parent	EB	EK	ER	EG	EW	ED	ET	UK	UR	US
EB	—	98	72	97	96	70	72	—	a	a
EK	87	—	64	92	91	82	86	—	a	a
ER	85	83	—	86	109	102	95	—	—	a
EG	90	93	90	—	101	82	82	a	a	a
EW	86	88	94	103	—	91	70	—	—	a
ED	73	84	93	80	76	—	83	a	a	a
ET	106	91	99	86	96	91	—	a	a	a
UK	—	b	a	a	a	a	8	—	89	1
UR	a	a	a	a	a	a	2	94	—	—
US	0	0	12	13	8	2	0	2	5	—

a—pollination attempted, no hybrid obtained.
b—see Vasek, 1958.

heterozygous cells in the Breckinridge plants is decidedly puzzling and will be discussed below.

In *C. unguiculata,* 526 metaphase cells from 22 plants of three populations were successfully analyzed. Two (out of seven) plants from Springville and one (out of 11) plant from Riverside were heterozygous for one translocation. The Kern Canyon population was structurally homozygous but the four plants examined had one or three supernumerary chromosomes. This population was grown from the same seed collection (*Lewis and Vasek 926*) as earlier Kern Canyon progenies, but supernumeraries had not previously been observed.

The major objective of cytological study of the hybrids was to determine their degree of structural heterozygosity and thus to ascertain the translocation differences between the parents. 2,013 cells from 177 plants of 55 hybrid combination were successfully analyzed.

In *Clarkia unguiculata* (excluding Springville, for now) the hybrids between UK and UR are either structural homozygotes or have one ring of four chromosomes indicating heterozygosity for one translocation. Therefore, these two populations have one genome in common and the Riverside population has a second genome differing from the first by one translocation. The genome that these populations have in common is the genome that Mooring (1958) designated as his standard "A" arrangement. The identity of this arrangement has been specifically tested, for I have made reciprocal hybrids between my Kern Canyon stock (*Lewis and Vasek 926*) and the Los Angeles stock (*Lewis 766*) used by Mooring. Both stocks and all hybrids tested (9 in number) were structural homozygotes (Vasek, unpublished). Since I can relate this study to Mooring's, at least in part, I will use other letter designations for genome arrangements of *C. exilis.*

Four genomes are found in *Clarkia exilis.* The genomes of Kern Canyon, Rancheria Road, Granite Station, and White River are all alike since all the hybrids between these populations are structural homozygotes and since their genomes react alike in hybrid combinations with Breckinridge Mountain, Deer Creek and Tule River (see table 4). The genome characteristic of these four populations will be designated arbitrarily as "L".

The Deer Creek and Tule River populations are both heterozygous for two genomes and since their genomes react similarly in hybrid combinations with the other populations, they are probably both heterozygous for the same two genomes. These genomes, arbitrarily designated "M" and "N", differ from "L" by one and two translocations respectively since the appropriate hybrids show one or two rings of four chromosomes.

Breckinridge Mountain also differs from "L" by two translocations as indicated by two rings of four chromosomes in the hybrids (table 4). Breckinridge Mountain similarly differs from "M" and "N" by one and two translocations respectively. The Breckinridge genome will be designated "P".

A second genome for Breckinridge Mountain (designated "Q"), and a fifth genome for *C. exilis,* can be inferred on several grounds. First, a ring of four chromosomes was observed, at whatever low frequency, in the parent greenhouse population. In addition, a ring of four and a ring of six were observed in earlier hybrids of Breckinridge Mountain with Kern Canyon and Rancheria Road (Vasek, 1958). Finally in the present hybrids between Breckinridge Mountain on one hand, and Kern Canyon, Rancheria Road or Tule River on the other, seven cells were observed with a ring or chain of six chromosomes. These cells are not reported in table 4 because they present a special problem and because several of these cells were not clear enough to be certain of interpretation. The low frequency of a chain or ring of six in these hybrids is roughly comparable to the low

TABLE 4. *Chromosome pairing in 10 parental greenhouse populations and in 55 hybrid combinations*

♀ \ ♂	EB	EK	ER	EG	EW	ED	ET	UK	UR	US
EB	9II	2r4	2r4	2r4	2r4	r4	2r4		a	a
EK	2r4	9II	9II	9II	9II	r4	r4 or 2r4		a	a
ER	2r4	9II	9II	9II	9II	r4 or 2r4	2r4			a
EG	2r4	9II	9II	9II	9II	r4	r4	a	a	a
EW	2r4	9II	9II	9II	9II	r4	r4			a
ED	r4	r4	r4	r4	r4	9II or r4	9II	a	a	a
ET	r4 or 2r4	r4 or 2r4	r4	r4	r4	r4	9II or r4	a	a	a
UK	—	b	a	a	a	a	r4 and ch8	9II or 1–3I	9II or r4	ch14
UR	a	a	a	a	a	a	r4 and ch4	9II	9II or r4	—
US	c	r4 and ch12	r4 and ch12	r4 and ch12	r4 and ch12	r4 and ch8	ch12	ch14	r4	9II or r4

a—no hybrid obtained.
b—r4, r8 or 2r4, r6 (see Vasek, 1958).
c—one hybrid plant obtained, no successful metaphase analysis.

frequency of a ring of four in the parent Breckinridge population.

A special problem arises in that cells with two closed rings of four were observed at a high frequency on the same slide with an occasional cell showing a ring or chain of six and a ring of four. (A check of old data indicates the same situation in the hybrids reported in 1958.) It is unlikely that the ring of six is in addition to the two rings of four since, considering the high frequency of two rings of four, one would expect to observe two rings of four plus part of the ring of six more frequently than only one ring of four plus the entire ring or chain of six. No combination of three catenations has ever been observed in these hybrids. It therefore seems likely that one of the closed rings of four is somehow capable of entering into formation of a ring of six. Some speculation is in order.

Since the maximum configuration is observed at a low frequency, the P and Q genomes of Breckinridge Mountain probably differ by relatively short chromo-

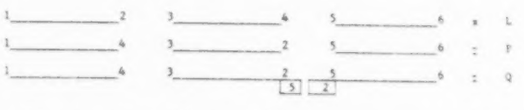

FIG. 1. Possible partial genome types found in the Kern Canyon (L) and Breckinridge Mountain (P, Q) populations of *Clarkia exilis*.

some segments. Since partial sterility is not involved, either in the parent Breckinridge population or in the hybrids, the structural difference between the P and Q genomes probably involves some sort of duplication, rather than a structural rearrangement per se. Otherwise, random segregation of bivalents would lead to deficiencies and probably, but perhaps not necessarily, to gametic sterility. Possible genome arrangements, based on the assumption that some sort of duplication seems probable, are shown in figure 1. In Q, the 2 arm has a small duplicated segment of (for example) the 5 arm (not necessarily terminal) and reciprocally the 5 arm has a small duplicated segment of the 2 arm. The "P" genome has no duplicated segments, but otherwise "P" and "Q" are identical. The PQ heterozygote would generally form only bivalents but rarely (depending on the length of the duplicated segment) synapsis and chiasma formation between the duplicated 2 segment and the 2 arm, and between the duplicated 5 segment and 5 arm would result in a ring of four. Similarly, in QL hybrids a ring of four (1.2, 2.3, 3.4, 4.1) would normally form. Rarely, synapsis and chiasma formation between the duplicated segments and the corresponding chromosome arm would add the 5.6 bivalent to the above ring of four, resulting in a ring of six. Of course, a second ring of four, which did not enter the immediately preceding discussion, also occurs in the QL hybrids giving the observed catenations of a ring of six and a ring of four or 2 rings of four. It should be clear that the above explanation is speculative in nature and not based on direct evidence. Since "Q" may not be strictly a translocation rearrangement, it will be considered a minor genome type and generally be omitted from further discussion.

Because closed rings of chromosomes have been observed in all hybrid combinations within *C. exilis*, the observed translocations are probably the maximum that occur. The translocations to be discussed next may not represent the maximum. In hybrids involving *C. unguiculata* mostly long chains of chromosomes have been observed (e.g., see figure 2). The maximum number of chromosomes involved in a long chain is open to question until a closed ring is observed. All that can be said is that a chain of, for example, 12 chromosomes indicates heterozygosity for at least five reciprocal translocations. It should also be pointed out that the long chains reported in table 4 were sometimes inferred, not observed. However, a chain of 12, for example, is justifiably inferred when cells with two chains of six, a chain of eight and a chain of four, a chain of nine and a chain of three are observed in the same plant. In all hybrids where long chains are reported, the maximum catenation was observed in, at most, only a few cells. Chain and ring formation, in hybrids with long chains, was highly irregular and comparable to the irregularity in an interspecific hybrid reported by the author in 1958.

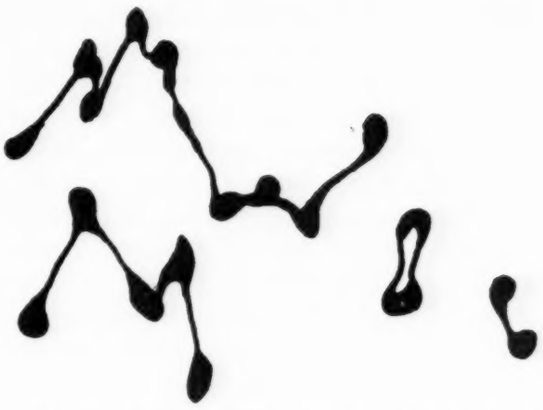

FIG. 2. Camera lucida drawing of a chain of 9, a chain of 5 and two pairs of chromosomes at first metaphase in the hybrid UK × US.

The Springville population (US) differs from Kern Canyon (UK) and Riverside (UR), and hence from Mooring's "A" arrangement, by six translocations as indicated by chains of 14 chromosomes in the hybrids (see table 4). One cell in one US-UR hybrid showed a closed ring of 14 indicating that six is probably the maximum number of translocation differences between US and the A arrange-

ment. The Springville genome will arbitrarily be designated "S."

The US population also differs from *C. exilis* by six translocations (r 4 and ch 12 in the hybrids). A possibility exists that ED differs from US by only five translocations, but the Springville parent itself was heterozygous for one translocation and the chain of 12 chromosomes observed in the hybrid may not be the maximum configuration. Of course, the ring of four and chain of 12 observed in other Springville-*exilis* hybrids may also not be the maximum.

On the basis of translocation differences, the Springville plants are equally different from both *C. unguiculata* and *C. exilis* and this probably explains why the hybrids are about equally sterile.

Plants having the "A" arrangement of *C. unguiculata* were successfully hybridized with *C. exilis* only when ET was used as the pollen parent. In these hybrids the maximum observed configuration was a ring of four and chain of eight, indicating heterozygosity for four translocations. This compares fairly well with the four translocations reported (Vasek, 1958) for the hybrid between sympatric Kern Canyon population of the two species. However, since EK and ET differ by one or two translocations, their hybrids with the same *C. unguiculata* should differ accordingly. This further suggests that the indicated translocation differences (or some of them) are not maximum and that one of those hybrids might show five translocations.

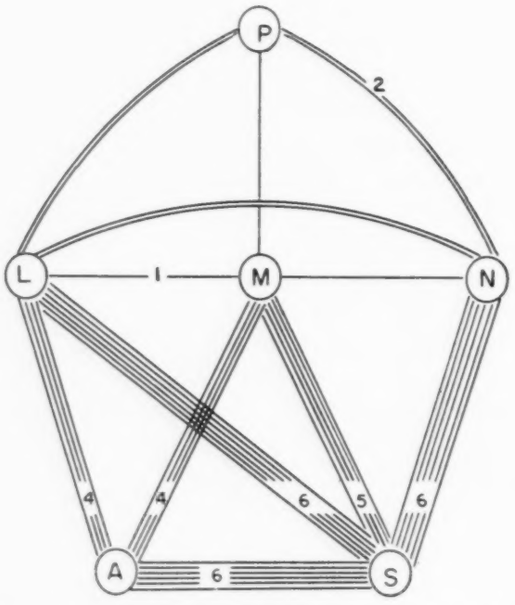

FIG. 3. Translocation differences between the indicated genomes (see text).

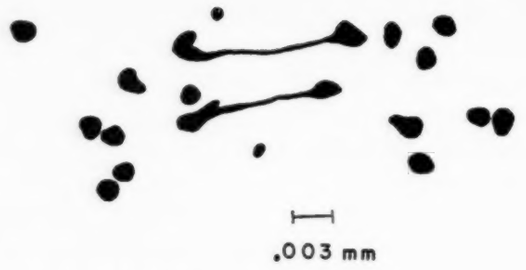

FIG. 4. Camera lucida drawing of a cell showing two bridges and two fragments at first anaphase in the hybrid US × EK.

A summary of the translocation differences between the various genomes will be found in figure 3.

In addition to the translocations, one hybrid between US and EK showed two bridges and two fragments at first anaphase indicating that these populations differ by two inversions (fig. 4).

DISCUSSION

Because of the limited range of *Clarkia exilis*, the pattern of translocations in that species is much simpler than the pattern found by Mooring (1958) in *C. unguiculata*. Nevertheless, the two patterns are generally similar. Both species have one genome arrangement that is widespread and more or less centrally located. In *C. unguiculata*, the "A" arrangement is widespread while four other genomes are found as homozygotes in sporadic, localized populations near the ecological limits of the species (Mooring, 1958). In *C. exilis*, the "L" arrangement is most common, being found as a homozygote over much of the range (four out of seven colonies). The other three genomes are found in the southernmost and the two northernmost colonies, thus being near the geographical and probably the ecological limits of the spe-

cies. Thus both species have several chromosome races and these races are roughly comparable to, but more regularly interfertile than, the well known chromosome races in *Datura* (Blakeslee *et al.*, 1937).

The widespread "A" arrangement of *C. unguiculata* was determined by Mooring to be ancestral to the other genomes of that species. One might therefore expect the widespread "L" genome to be ancestral to other arrangements of *C. exilis*. However, by applying the criterion of simplicity, as was done by Mooring, it seems more probable that the "M" genome of the northern colonies of *C. exilis* is ancestral. From the "M" genome, "L," "N" and "P" can each be derived by only one translocation. That the most common genome of *C. exilis* was probably derived from one that is now marginal to the center of distribution is one important difference in the pattern of chromosome arrangements in the two species. Another cytological difference is that 35% of the wild plants of *C. unguiculata* sampled by Mooring were structural heterozygotes whereas I found structural heterozygotes only in a few greenhouse populations of *C. exilis*.

The Springville population has not yet been fitted into the pattern of either species and indeed it is difficult to do so. The Springville plants are intersterile with both species and differ chromosomally from both species by about six translocations. Despite the fact that other genomes of *C. unguiculata* differ from "A" by only one or two translocations, the morphological identification (but not identity) of the Springville plants with *C. unguiculata*, suggests that the Springville population is an extreme chromosome race of that species. The Springville population is in a habitat that I (but not Mooring) consider fairly typical for *C. unguiculata*, being neither geographically nor ecologically marginal. Morphologically and ecologically, then, the Springville plants can be referred to *C. unguiculata*, but chromosomally and genetically they belong to neither species. Perhaps the best interpretation is to consider the Springville plants as an incipient species.

The present study suggests at least three broad stages in the formation of diploid *Clarkia* species. The first stage, chromosome differentiation on the order of one or two translocations, is commonly attained. The chromosome races resulting from populations becoming homozygous for various rearrangements have not accumulated any significant fertility barriers or morphological differences.

The Springville population represents, perhaps, a middle stage in which strong chromosome differentiation, on the order of four to six translocations, has been accomplished along with strong barriers to hybridization and almost complete sterility barriers. Accumulation of morphological differences, however, has occurred only in a rudimentary way. The morphological features of the Springville plants, although sufficient to call attention to this population and warrant its inclusion in the present study, are still not different enough from *C. unguiculata* for most taxonomists to recognize a separate species.

The last stage is represented by *Clarkia exilis*, in which strong chromosomal differentiation and essentially complete sterility barriers are combined with sufficient morphological differences to make that species distinct from *C. unguiculata* (Vasek, 1958). The morphological divergence between the two species is not extreme, the differences being mostly quantitative with no one single clear cut key character to separate *C. exilis* from all forms of *C. unguiculata*. Many taxonomists would no doubt hesitate to recognize *C. exilis* as a distinct species. Nevertheless, the summation of several quantitative characters gives *C. exilis* a distinctly different appearance from *C. unguiculata*, especially when the two grow sympatrically. Furthermore, the seven colonies determined as *C. exilis* on morphological grounds are all interfertile with each other, are all equally intersterile

with *C. unguiculata* and are all chromosomally quite distinct from *C. unguiculata*.

In the first stage of species formation described above, all the chromosomes involved in translocations are included in the catenations formed in translocation heterozygotes. Alternate disjunction is regular, resulting in spores without deficiencies or duplications. Hence, there is no chromosomal mechanism for hybrid sterility.

In the middle and last stages of species formation, chromosome repatterning is more extreme. The catenations formed in translocation heterozygotes usually do not include all the chromosomes involved with translocations. In fact, pairing and ring formation are quite irregular and disjunction is also quite irregular leading to deficiencies in spores. Meiotic irregularities thus explain all, or at least most, of the hybrid sterility. The large cytological difference between the first and the middle stage suggests either another stage in between or a very rapid, irreversible transition between the two. Since extreme chromosome repatterning appears to be a major cause of hybrid sterility, it seems likely that there is one rapid transition from the first to the middle stage. A mutator genotype which persists only a short time and which causes many chromosome breakages, may rapidly produce an occasional extensively reorganized genome that is adapted to an available habitat. This hypothesis, together with supporting evidence from several sources, has been amply developed by Lewis and Raven (1958, p. 332) and need not be discussed further here.

Determining the origin of *C. exilis* cannot be done with a great deal of confidence. However, several clues are available. Obtaining hybrids between *C. exilis* and *C. unguiculata* is generally difficult, but ten hybrids were obtained between Tule River plants of *C. exilis* and plants having the "A" arrangement of *C. unguiculata*. All other combinations between *C. exilis* and the "A" arrangement failed. This suggests that the Tule River population is genetically closer to, or at least has less strongly developed genetic barriers to crossing with, the "A" arrangement of *C. unguiculata* than are other populations of *C. exilis*. Other supporting evidence is that the probable ancestral genome of *C. exilis* is found in the Deer Creek and Tule River populations. Further, there is some tendency for these populations to resemble *C. unguiculata* in morphological features of the flower, particularly those relating to the pollinating system (Vasek, 1958).

The Springville population again enters into the problem. The Springville plants cross as readily with plants having the "A" arrangement of *C. unguiculata* as do different "A" populations with each other. This suggests that genetically the Springville plants are quite close to *C. unguiculata*. On the other hand, hybrids were also obtained between Springville and all the colonies of *C. exilis*, but with more difficulty. On this basis, *C. exilis* is genetically closer to Springville than to the "A" arrangement of *C. unguiculata*. Chromosomally the three types are equally distinct and equally intersterile, but the Tule River population may be more similar to Springville, by one translocation, than are other colonies of *C. exilis*.

Thus, it seems that the Springville population may be segregating, or may have recently segregated, from a stock of *C. unguiculata* that has the capacity from time to time to undergo extensive reorganization of chromosomes and which is similar to, or perhaps the same as, the stock from which *C. exilis* diverged. The close geographical proximity of Springville to the probable ancestral type of *C. exilis* at the Tule River (a distance of eight airline miles) suggests that the Tule River basin is a possible geographic origin of *C. exilis*.

The genetic origin of *C. exilis* is still, and probably will remain, in doubt. However, derivative species of *Clarkia* are generally found adjacent to the parental species (Lewis and Raven, 1958) indicating recent evolution subsequent to

any major migration. Considering, then, the close genetic (on the basis of crossability) and geographic relationship between *C. exilis* and Springville, it appears likely that forms of *C. unguiculata,* directly descended from the population which gave rise to *C. exilis* and to Springville, may still be located in the vicinity of the Tule River. The hypothesis presented here, namely, that the genetic origin of *C. exilis* was in a form of *C. unguiculata* near the Tule River basin, might be strengthened by testing crossability of *C. exilis* and Springville with populations of *C. unguiculata* now growing in or near the Tule River basin.

It is thus envisioned that a form of *C. unguiculata* in which translocations may occur rapidly (Lewis and Raven, 1958) gave rise by several translocations to a form represented today by the Tule River population of *C. exilis.* Then a minor radiation of chromosome races resulted in the present distribution of four genome types, one of the secondary genomes being the widespread "L" genome and the ancestral genome remaining in the two northernmost localities. A similar process gave rise to the Springville population but the latter has not progressed beyond an intermediate stage in the process of species formation.

Summary

Seven populations of *Clarkia exilis* and three populations of *C. unguiculata* were intercrossed in all combinations. Fertile hybrids were easily obtained within *C. exilis.* These hybrids indicated that the parent populations differed by from zero to two reciprocal translocations. Four genome arrangements were identified. One of the genomes from the northern populations was determined as probably ancestral to the other three genomes. The pattern of translocations was compared with the pattern reported (Mooring, 1958) for *C. unguiculata.* Within *C. unguiculata,* two populations were determined to have the "A" chromosome arrangement and the third population (Springville) was determined to differ from both species by six translocations. Populations with the "A" arrangement were fully interfertile. These populations were easily crossed with Springville, but the hybrids were sterile. Hybrids between *C. exilis* and plants with the "A" arrangement of *C. unguiculata* were produced, with difficulty, in only one combination and were sterile.

C. exilis and *C. unguiculata* are chromosomally, gentically, and morphologically somewhat similar, but distinct. The similarity suggests that morphological differentiation is the last step in species formation. Springville, since it is morphologically identified with *C. unguiculata,* but chromosomally and genetically well differentiated, is interpreted as an incipient species. A model for formation of diploid *Clarkia* species is suggested. Possible genetic and geographic origins of *C. exilis* are discussed.

Acknowledgments

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EVOLUTION OF GAMETOPHYTIC AND SPOROPHYTIC SYSTEMS OF SELF-INCOMPATIBILITY IN ANGIOSPERMS

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Self-incompatibility has been found in at least 78 angiosperm families and occurs in every major phylogenetic line (East, 1940; Fryxell, 1957; Brewbaker, 1957). It has even been discovered in at least three gymnosperm species (*Pinus sylvestris*—Gustaffson, cited by Bateman, 1952; *Larix laricina, Pseudotsuga taxifolia*—Fryxell, 1957), and one pteridophyte, *Pteridium aquilinum* (Wilkie, 1956). It occurs not only in hermaphrodite species, but also in monoecious species with unisexual flowers on the same plant (Godley, 1955).

Genetically, self-incompatible species have been divided into two groups, gametophytic and sporophytic. In each of these systems there are variations particularly with regard to (i) the number of **S** loci and (ii) the interaction relationship between the alleles at the same or different loci (Pandey, 1957). Although there is no family yet known which has been found to contain both gametophytic and sporophytic genetic systems in the different species of the same family, this possibility is strongly suggested as will be evident from the following discussion.

The evolution of the self-incompatibility phenomenon has been discussed generally by several authors (Whitehouse, 1950; Lewis, 1954; Stebbins, 1957). The present paper deals particularly with the evolution of the different genetic systems of self-incompatibility in angiosperms. The physiological and genetical mechanisms of self-incompatibility systems have evolved in association with several variable phenomena. Some of these phenomena which will be discussed in this paper are: (i) pollen cytology; (ii) time of nuclear action; (iii) production of pollen growth substance; (iv) cytokinesis in the pollen mother cell; and (v) site of inhibition.

POLLEN CYTOLOGY

In angiosperms when mature pollen grains are released from the anthers they are either binucleate or trinucleate. The number of nuclei in mature pollen grains is constant in the genus (Schnarf, 1937). In a large number of families the number is constant for the whole family (tables 1 and 2). However, there are 13 families which are known to contain self-incompatible species and in which both binucleate and trinucleate pollen grains are found in the different genera of the same family (table 3).

Recently Brewbaker (1957) claimed that there was a close relationship between the two types of pollen and the two types of self-incompatibility—gametophytic and sporophytic. The gametophytic species had binucleate pollen grains and the sporophytic species trinucleate grains. However, there are some exceptions which remained unexplained. The three graminaceous species studied for

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TABLE 1. *Cytokinesis and suggested system of self-incompatibility in homomorphic angiosperm families in which self-incompatibility has been known to occur and which have binucleate pollen grains only*

Families	Cytokinesis in pollen mother cells	System(s) of self-incompatibility
Dicots—31 families		
Nymphaeaceae	Both simultaneous and successive	Gametophytic only
Bombacaceae, Cistaceae, Resedaceae	Not known	
Acanthaceae, Aceraceae, Anonaceae, Begoniaceae, Betulaceae, Bignoniaceae, Convulvulaceae (excluding Cuscutaceae), Cornaceae, Ericaceae, Fagaceae, Goodeniaceae, Hamamelidaceae, Hypericaceae, Leguminosae,* Moraceae, Myrtaceae, Onagraceae,* Plantaginaceae, Polemoniaceae, Ranunculaceae, Rosaceae,* Saxifragaceae, Scrophulariaceae,* Solanaceae,* Theaceae, Tropaeolaceae, Violaceae	Simultaneous	
Monocots—7 families		
Orchidaceae	Both simultaneous and successive	Gametophytic only
Iridaceae	Simultaneous	
Agavaceae, Amaryllidaceae, Bromeliaceae, Commelinaceae,* Zingiberaceae	Successive	

* In these families the system of self-incompatibility has been studied.

TABLE 2. *Cytokinesis and suggested system of self-incompatibility in homomorphic angiosperm families in which self-incompatibility has been known to occur and which have trinucleate pollen grains only**

Families	Cytokinesis in pollen mother cells	System(s) of self-incompatibility
Dicots—10 families		
Apocynaceae	Both simultaneous and successive	May have both systems depending on the type of pollen cytology
Lentibulariaceae	Not known	
Caprifoliaceae, Chenopodiaceae, Compositae,† Cruciferae,† Geraniaceae, Nyctaginaceae, Portulacaceae, Thymeliaceae	Simultaneous	Sporophytic only
Monocot—1 family		
Gramineae†	Successive	Gametophytic only

* Dioscoreaceae (monocot) has simultaneous cytokinesis but pollen cytology is not known.
† In these families the system of self-incompatibility has been studied.

TABLE 3. *Cytokinesis and suggested system of self-incompatibility in homomorphic angiosperm families in which self-incompatibility has been known to occur and which have both binucleate and trinucleate pollen grains in the same family*

Families	Cytokinesis in pollen mother cells	System(s) of self-incompatibility
Dicots—11 families		
Asclepiadaceae, Campanulaceae, Euphorbiaceae, Labiatae, Lobeliaceae, Loganiaceae, Malvaceae, Papaveraceae,* Passifloraceae, Rutaceae, Sterculiaceae*	Successive	Gametophytic only
	Simultaneous	Both gametophytic and sporophytic may occur in the same family, depending on the type of pollen cytology in the individual species
Monocots—2 families		
Araceae	Successive	Gametophytic only
Liliaceae	Both successive and simultaneous	May have both systems in the same family depending on the types of pollen cytology and cytokinesis in individual species

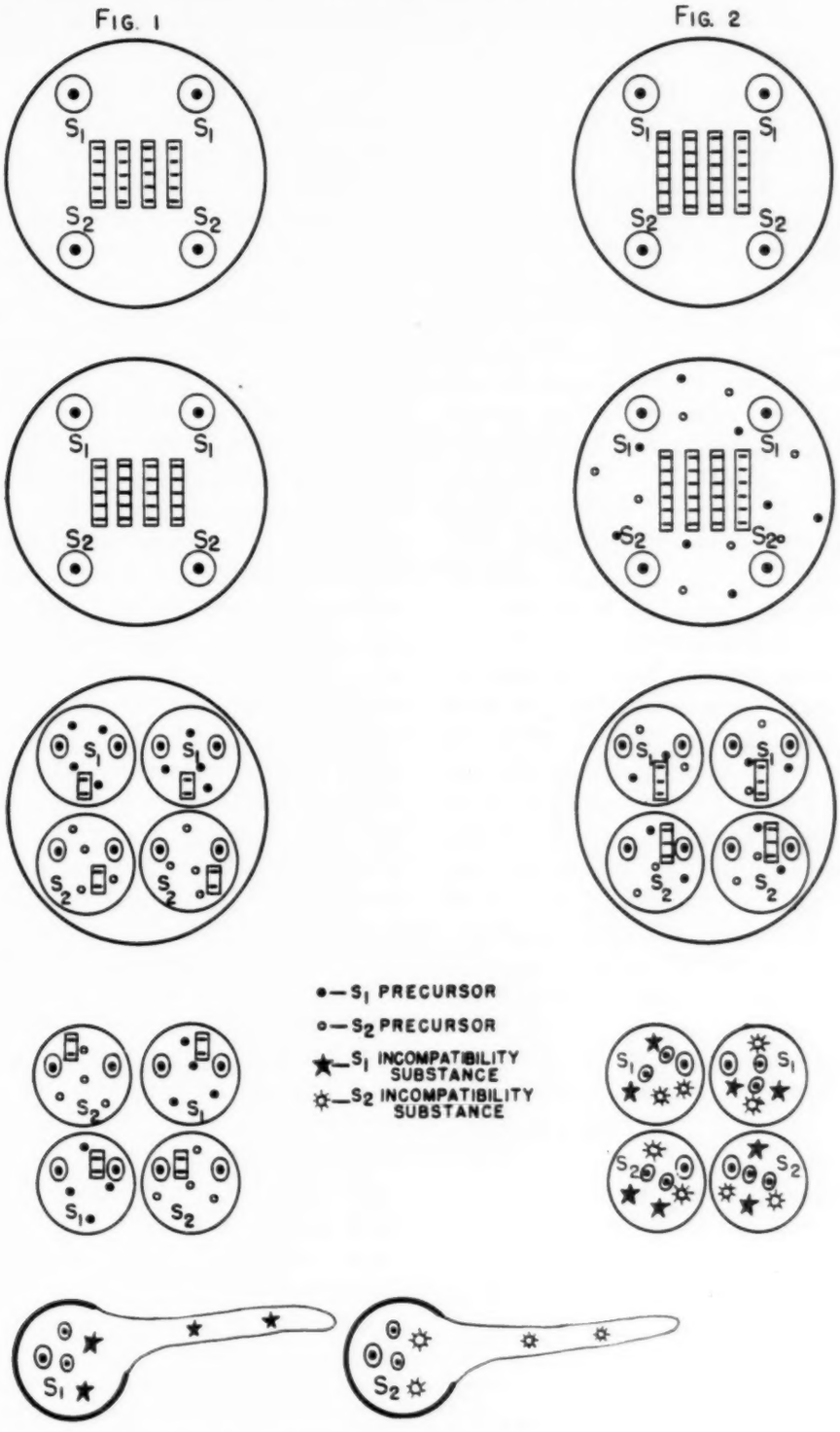
* In these families the system of self-incompatibility has been studied.

self-incompatibility, *Secale cereale*, *Festuca pratensis* (Lundqvist, 1954, 1955) and *Phalaris coerulescens* (Hayman, 1956), have a gametophytic system but have trinucleate grains. The whole relationship appears to break down with heteromorphic species which are sporophytic but which may have binucleate (*Primula* and *Lythrum*) or trinucleate (*Linum* and *Fagopyrum*) pollen grains.

There is another relationship, that between pollen cytology and the place of inhibition (Brewbaker, 1957), which seems to be more consistent in the various species examined. In species with binucleate pollen grains there is generally very little, if any, inhibition of pollen germination, major inhibition occurring at the pollen tube stage. In trinucleate pollen species, however, inhibition occurs at the pollen germination stage; the majority of pollen grains remain ungerminated and the few which germinate are unable to penetrate the stigma. Most of the heteromorphic and homomorphic species are consistent in this respect. However, in *Helianthus annuus* (Habura, 1957), which is trinucleate, the inhibition occurs mostly in the style and in *Beta vulgaris* (Savitsky, 1950) which also has trinucleate grains, inhibition occurs in the ovary. That there is some relationship between gametophytic and sporophytic systems, pollen cytology and place of inhibition is beyond doubt. However, the causal mechanism of this relationship and of a number of exceptions to it is not understood.

TIME OF THE S ALLELE ACTION

The difference between the gametophytic and sporophytic systems of self-incompatibility lies in the time at which **S** alleles act to produce incompatibility substances or their precursors which later change into incompatibility substances (Lewis, 1956; Pandey, 1958*a*, *b*). Pandey (1958*a*) suggested that the time of the **S** allele action in the sporophytic system is after anaphase II in the pollen mother cell, before the separation of the four microspores from the common cytoplasm. In the gametophytic system the time of **S** allele action is after cytokinesis (fig. 1). When the **S** gene action occurs before cytokinesis, the products of the different **S** alleles in the four nuclei are able to



FIGS. 1 AND 2. Hypothetical relationship between the amount of available metabolites in the pollen mother cell (PMC), time of nuclear activity (**S** gene action and pollen cytology) and time of conversion of precursors into incompatibility substances. The amount of metabolites are shown by four blocks in each PMC, each block having either five (fig. 1) or six (fig. 2) units. Each mitotic division is assumed to require three units of metabolites. FIG. 1. Gametophytic: Low metabolite content in the PMC leads to late **S** gene action after cytokinesis and late precursor conversion after pollen germination. FIG. 2. Sporophytic: High metabolite content in the PMC leads to precocious nuclear action before cytokinesis and precocious precursor conversion before pollen germination. For details see text.

diffuse into the common cytoplasm (fig. 2). The specific substances in the sporophytic system are, therefore, already present in the cytoplasmic material which forms the microspore wall. These substances produce the incompatibility reaction on contact of the pollen grains with incompatible stigmata thus inhibiting the pollen germination. In the gametophytic species the specific substances are produced internally within each microspore whose wall is free of specific substances. Hence, in these species, pollen grains germinate and penetrate the incompatible style, but the growth of pollen tubes is stopped after the specific substances of the pollen tube and the corresponding substances of the style are brought together through diffusion or other processes.

The above hypothesis amounts to precocious nuclear activity in the sporophytic system. This is supported by the fact that gametophytic species, in which **S** allele action is delayed, also have delayed mitotic division of the generative nucleus in the microspore, giving only binucleate pollen grains. In the sporophytic species, which have precocious action of the **S** alleles, both mitotic divisions of the microspore take place before pollen maturity, thereby giving the trinucleate grains. Thus with this hypothesis, there is a relationship between the *time of* ***S*** *allele action, the time of the second microspore division* (pollen cytology) and the *site of inhibition* (fig. 3A).

It has been stated before that, while the relationship between the three phenomena—type of incompatibility reaction, pollen cytology and site of inhibition—is fairly consistent in homomorphic species, it holds good in heteromorphic species only in relation to pollen cytology and site of inhibition. The heteromorphic species, though all sporophytic, may have binucleate pollen and inhibition of pollen tube growth in the style, or trinucleate pollen and inhibition of pollen germination at the stigma. It is not understood, however, how some heteromorphic species which have sporophytic system can produce inhibition in the pollen tube growth instead of, as in other sporophytic species, in pollen germination. This inconsistency is explained if it is assumed that **S** alleles produce only precursors, and there is a difference in the time of the change of precursors into incompatibility substances. In trinucleate grains the precursor conversion takes place in the microspores, whereas in the binucleate grains it occurs during pollen tube growth. The trinucleate grains, with their specific incompatibility substances, would thus be ready to react with the correspondingly specific incompatibility substances on the stigma, and would be stopped from germination. The binucleate grains would lack incompatibility substances and the inhibition would occur only when the precursors in the pollen tube are changed into incompatibility substances and react with their counter-parts in the style.

The physiological relationship between pollen cytology and the conversion of precursors into incompatibility substances may lie in the cytoplasmic nutritional condition of the pollen mother cell or microspore. It is possible that the conversion of the precursors into incompatibility substances occurs only at a relatively low concentration of available metabolites, a high concentration of the latter being toxic to this presumably enzymatic conversion reaction. This view is supported by the experiments of Eue (1953) who observed that the specific inhibition reaction is dependent on factors of nutrition physiology. Eue crossed self-sterile *Petunia* with one of its mutants called defecta (d), characterized by strong chlorophyll deficiency in the leaves. It was found that pollen tubes of the smaller d-pollen grains with less metabolites were inhibited more during their growth through the style than the normal pollen tubes containing the same **S** alleles but having more reserve substances. Similarly self-fertility of the selfed progeny of the naturally occurring or induced pseudo-self-fertile plants of several species decreases considerably, and this has been

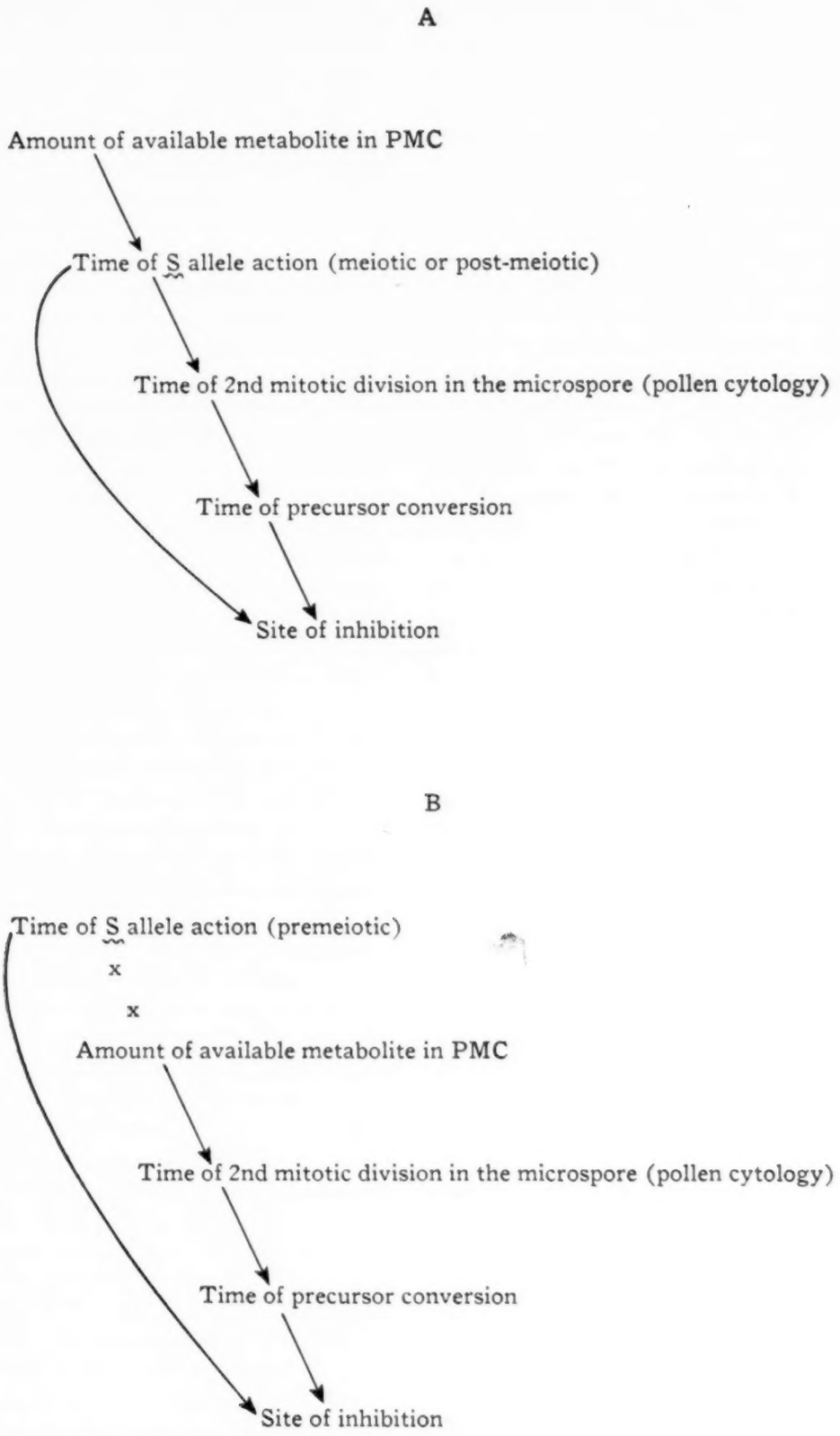

A
Amount of available metabolite in PMC
Time of S allele action (meiotic or post-meiotic)
Time of 2nd mitotic division in the microspore (pollen cytology)
Time of precursor conversion
Site of inhibition
B
Time of S allele action (premeiotic)
x
x
Amount of available metabolite in PMC
Time of 2nd mitotic division in the microspore (pollen cytology)
Time of precursor conversion
Site of inhibition

FIG. 3. Relationship in the sequence of events in the expression of incompatibility. x x—no relationship; →—causal relationship. A. Homomorphic system: There is direct causal relationship between the five factors. B. Heteromorphic system: The direct causal relationship is limited to the last four factors. The time of S allele action affects the site of inhibition in both systems. For details see text.

ascribed to an increase in the true self-incompatibility resulting from inbreeding and not to the inbreeding depression *per se* (Myers, 1945; Adams, 1953; Pandey, 1956*a*). It is possible, therefore, that the metabolite-deficient condition of the cytoplasm in the trinucleate grains, in which two mitotic divisions have occurred, favors a rapid conversion of precursors into incompatibility substances, while it is stopped or is very slow in the relatively metabolite-rich binucleate grains.

That the division of the generative nucleus too may be dependent on the available amount of metabolite in the microspore, is suggested from the experiments of Poddubnaya-Arnoldi (1936) and Eigsti (1941). Working on several species they found that in pollen grains which are normally shed at the binucleate stage, the generative nucleus could be made to divide before germination, if the grains were allowed to lie for a time on a sugar-agar substrate. Furthermore, as Schürhoff (1926) also pointed out, the trinucleate pollen condition is very often accompanied by the development of anther periplasmodium, viz. in Compositae, Geraniaceae, Caprifoliaceae, Amaranthaceae, Moringaceae and others among dicots; and Aponogetonaceae, Araceae, Alismataceae, Butomaceae, Lemnaceae and others among monocots. Since the tapetal periplasmodium is considered to serve a nutritive function for the development of microspores, its association with the trinucleate condition of the pollen is significant. These observations strongly indicate that the time of second microspore division, the time of conversion of precursors into incompatibility substances and the site of inhibition are dependent basically on one factor—lack or abundance of available metabolites in the microspore—which controls the whole chain of events. Since the time of second microspore division is dependent also, as has been suggested earlier, on the precocious nuclear activity (**S** allele action) in the microspore mother cell, it seems that the precocity of nuclear action may itself be dependent on the excess of metabolites in the microspore mother cell and this triggers the chain reaction.

The selection for a critical amount of available metabolites in the microspore mother cell may, therefore, have been one of the basic physiological mechanisms in the evolution of self-incompatibility systems. A slight excess of metabolite in the pollen mother cell would trigger a chain reaction which would follow a pattern associated with the sporophytic system of incompatibility, viz. precocious nuclear action (**S** gene action), precocious second mitotic division in the microspore giving trinucleate pollen grains at maturity, lowering the available metabolite content in the microspore to a critical minimum which allows the conversion of precursors into incompatibility substances and inhibition of pollen germination at the stigma (figs. 2 and 3). A critically lower concentration of metabolites in the pollen mother cell would trigger an alternative chain of events associated with gametophytic incompatibility, viz. delayed second mitotic division in the microgametophyte, thus giving binucleate pollen grains at maturity, excess of available metabolite content in the microspore causing lack or slower conversion of precursors into incompatibility substances, and inhibition of pollen tubes in the style or ovary (figs. 1 and 3).

Pollen Growth Substance

The suggestion that the cytoplasmic constituents control the time of **S** allele action is further supported by the experiments of Crowe (1955) with *Oenothera* hybrids. Interaction between **S** alleles in the pollen has been found to occur in hybrids between gametophytically self-incompatible *O. pallida* ($\mathbf{S}_{1,2}$) and self-compatible *O. trichocalyx* ($\mathbf{S}_{t,t}$) with the latter as the female parent. There is no competition interaction between the **S** alleles in the reciprocal cross when the cytoplasm is contributed by self-incompatible *O. pallida*. In the light of the preceding hypothesis it may be suggested

that the **S** gene action in *O. pallida* cytoplasm occurs after cytokinesis in the individual microspores whereas in *O. trichocalyx* cytoplasm, presumably due to some cytoplasmic condition, it occurs precociously before cytokinesis. This would produce competition interaction between the alleles in *O. trichocalyx* cytoplasm but not in *O. pallida* cytoplasm.

Figure 4 illustrates the physiology, suggested by the present author, of the self-incompatibility reaction in gametophytic and sporophytic systems respectively, with particular reference to the time of action of the two sub-units of the **S** gene concerned with specific pollen growth and pollen incompatibility substances. In the gametophytic species, non-specific precursors of pollen growth substance would be present in the pollen mother cells. The action of the **S** allele sub-units governing the production of specific pollen growth substance and the specific incompatibility precursors, the latter being superimposed upon the former (Lewis and Crowe, 1958), takes place in individual microspores after cytokinesis (fig. 4A). In the sporophytic species the production of the specific pollen growth substance and the specific incompatibility precursors occurs before cytokinesis in the pollen mother cell (fig. 4B).

In the *Oenothera* hybrid with *O. trichocalyx* cytoplasm, discussed above, only the production of the specific pollen growth substance is precocious and takes place before cytokinesis. The precursors of the incompatibility substances are produced, however, as usual for the gametophytic system, after cytokinesis. This is shown by the absence of the interaction between the sub-units of the two **S** alleles controlling pollen specificity. The variation in the time of action of the two sub-units of the **S** gene produces pollen grains which contain some inappropriate specific pollen growth substance (not specific to the **S** allele contained by them) which would not have the specific incompatibility precursors superimposed upon them. These "naked" pollen growth substances are presumably inactivated on a style carrying any **S** allele. Considering that the dominance relationship between the **S** gene sub-units controlling the pollen growth substance is the same as that in the corresponding sub-units controlling the incompatibility substance, this would result in two phenomena: (i) All pollen grains having *O. trichocalyx* cytoplasm will have a lower amount of growth substance and hence will have a lower rate of pollen tube growth than those with *O. pallida* cytoplasm. (ii) The two types of pollen grains with different **S** alleles, having *O. trichocalyx* cytoplasm will also have different rates of pollen tube growth. These results have been observed in *Oenothera* hybrids (fig. 4C).

The hypothesis that naked pollen growth substance is inactivated on a style carrying any **S** allele has a particular significance in relation to the crossing behavior between self-compatible (SC) and self-incompatible (SI) species (Lewis and Crowe, 1958); ♀ SC × ♂ SI is successful but the reciprocal, ♀ SI × ♂ SC, is unsuccessful. The pollen grains of long established self-compatible species have lost their incompatibility specificity which was superimposed upon their pollen growth substance and are thus incapable of growing on self-incompatible styles.

It may be further concluded that the qualitative characteristics of the expression of incompatibility reaction are under the control of the **S** gene complex, while the time of the actual triggering of the physiological mechanism and its extent of reaction are controlled by certain cytoplasmic constituents of the species (which may be controlled polygenically).

Cytokinesis in the Microsporogenesis

The type of cytokinesis in the microsporogenesis of a species has an important bearing on the type of self-incompatibility system—gametophytic or sporophytic—that the species will have. There are two types of cytokinesis of the pollen mother cell—successive and simultaneous.

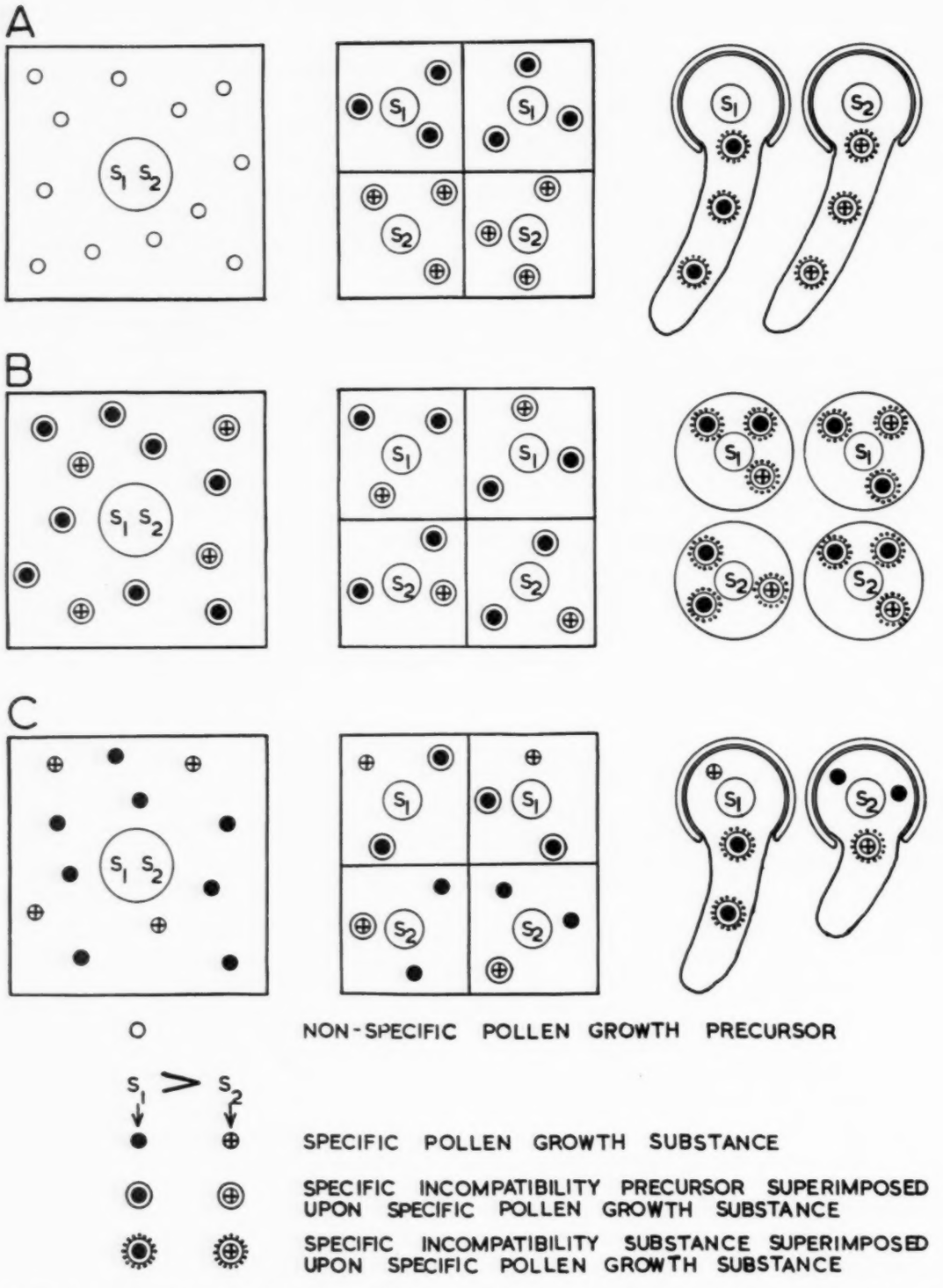

A
B
C
NON-SPECIFIC POLLEN GROWTH PRECURSOR
SPECIFIC POLLEN GROWTH SUBSTANCE
SPECIFIC INCOMPATIBILITY PRECURSOR SUPERIMPOSED UPON SPECIFIC POLLEN GROWTH SUBSTANCE
SPECIFIC INCOMPATIBILITY SUBSTANCE SUPERIMPOSED UPON SPECIFIC POLLEN GROWTH SUBSTANCE

FIG. 4. Physiology of self-incompatibility reaction with particular reference to the time of action of the two sub-units of the S gene concerning specific pollen growth and pollen incompatibility substances. A. Gametophytic: Both units of the S gene act after cytokinesis. B. Sporophytic: Both units of the S gene act before cytokinesis. C. *Oenothera* hybrid with *O. trichocalyx* cytoplasm: specific pollen growth substance unit acts precociously before cytokinesis in the PMC while specific incompatibility precursor unit acts, as normal for gametophytic action, after cytokinesis. For details see text.

In the successive type of cytokinesis a cell plate is laid down after each of the two meiotic nuclear divisions. In the simultaneous type the pollen mother cell is divided into four parts after both meiotic nuclear divisions are over. Although either the simultaneous or the successive mode of cytokinesis may tend strongly to predominate in certain groups of plants, such as simultaneous in dicots and successive in monocots, there are so many exceptions to this rule and so much variation that the character is of very restricted taxonomic value (Sharp, 1934). Several experiments have suggested that the differences in the modes of cytokinesis are due to modification caused by cytoplasmic (Yasui, 1931), temperature or other external influences (Sakamura and Stow, 1926).

In species with simultaneous cytokinesis, the precocious nuclear action in the pollen mother cell will give trinucleate pollen grains and sporophytic incompatibility and the delayed nuclear action will give binucleate pollen grains and gametophytic incompatibility (fig. 5, A and B). However, in the species with successive cytokinesis, the precocious nuclear action producing trinucleate pollen grains will have

Fig. 5

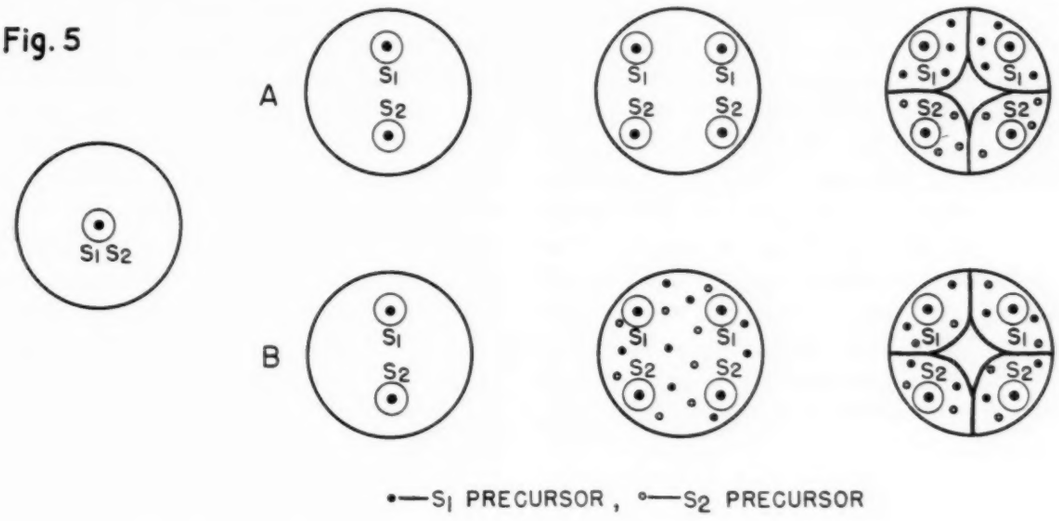


Fig. 6

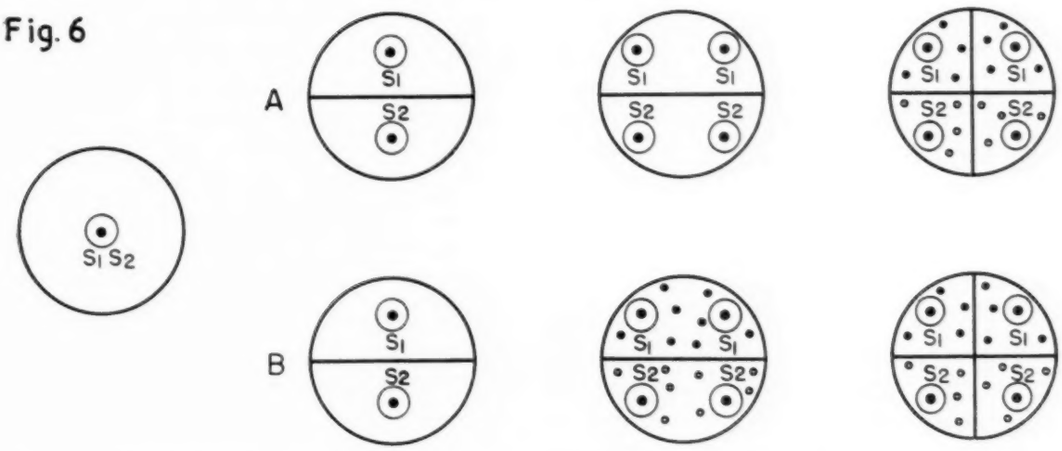


FIG. 5. Simultaneous cytokinesis and pollen reaction. The incompatibility reaction of pollen grains is gametophytic when the **S** gene action is delayed (4A) and sporophytic when it is precocious (4B).

FIG. 6. Successive cytokinesis and pollen reaction. The incompatibility reaction of pollen grains is gametophytic whether the **S** gene action is delayed (3A) or precocious (3B).

no effect on the incompatibility reaction of the pollen grains. In this type of cytokinesis, after the heterotypic nuclear division, the pollen mother cell divides into two cells by a partition wall. Since after the homotypic nuclear division the two nuclei in each of the diad cells—assuming the **S** locus is very close to the centromere thus barring second division segregation—are similar in **S** gene composition, the precocity of the **S** gene action is ineffective and the incompatibility reaction of pollen remains gametophytic. Each quartet of microspores produces physiologically two kinds of pollen grains in equal numbers (fig. 6, A and B). Thus in species with successive cytokinesis of the pollen mother cells, the trinucleate condition of the pollen grain is not accompanied by sporophytic determination of the incompatibility reaction (fig. 7). This is precisely what may be happening in Gramineae which has successive cytokinesis in the microsporogenesis. The three gramineaeceous species studied, although trinucleate, have gametophytic incompatibility and the inhibition occurs at the pollen germination stage as would be expected on the basis of the preceding discussion.

Among the homomorphic families the three sporophytic families studied (Compositae, Cruciferae and Sterculiaceae) have, as expected, the simultaneous type of division only. Six other families which have been investigated have simultaneous division and have a gametophytic system (Solanaceae, Leguminosae, Scrophulariaceae, Onagraceae, Papaveraceae and Rosaceae). The two monocot families studied (Commelinaceae and Gramineae) have successive cytokinesis and thus could only have a gametophytic system. Both families have, as expected, gametophytic systems.

If the time of **S** allele action in heteromorphic families is the same as in other sporophytic homomorphic species (i.e., during meiosis of microsporogenesis) and if all of them have sporophytic incompatibility, as is suggested by the seven heteromorphic families which have been studied, then all heteromorphic families should have only simultaneous cytokinesis. Of the 12 heteromorphic families (table 4), for which data are available (East, 1940; Bateman, 1954; Baker, 1948; 1956) eleven are dicots and have simultaneous cytokinesis and one, Pontederiaceae, is a monocot and has successive cytokinesis (Schnarf, 1931; Palm, 1920). On the above assumption of time of **S** allele action, Pontederiaceae should have gametophytic incompatibility. However, it is very likely that this family, like the heteromorphic families studied, would also

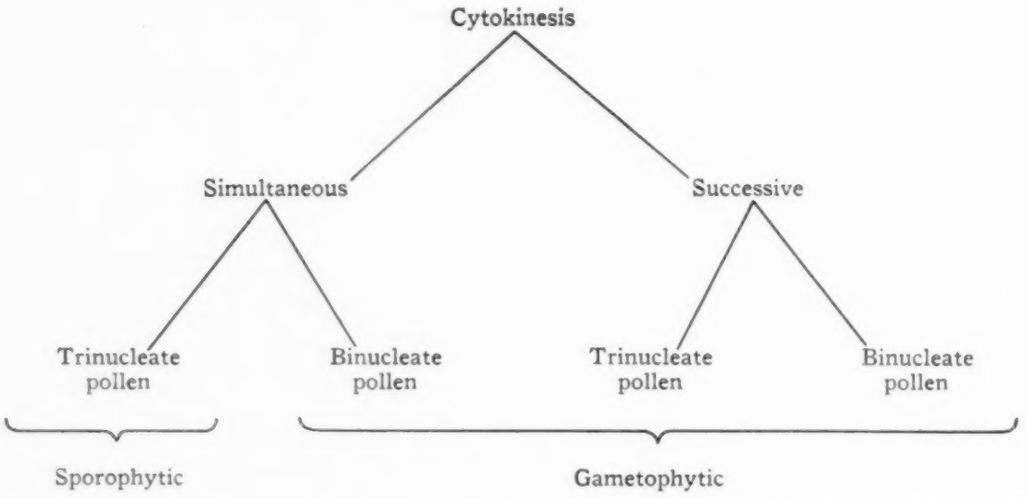


FIG. 7. Genetic system of self-incompatibility in homomorphic species in relation to the cytokinesis in the pollen mother cells and pollen cytology.

TABLE 4. *Pollen cytology, cytokinesis and suggested system of self-incompatibility in heteromorphic angiosperm families in which self-incompatibility has been known to occur**

Binucleate pollen	Trinucleate pollen	Bi- and trinucleate pollen	Cytokinesis in pollen mother cells	System(s) of self-incompatibility
Dicots—3 families	Dicots—4 families	Dicots—5 families		
Boraginaceae (including Heliotropiaceae)†	Linaceae†	Gentianaceae	Simultaneous	Sporophytic only
Lythraceae†	Oxalidaceae†	Oleaceae†		
Primulaceae†	Plumbaginaceae	Polygonaceae†		
		Rubiaceae		
		Verbenaceae ?		
	Erythroxylaceae		Not known	
Monocot—1 family				
Pontederiaceae			Successive	Gametophytic ? or sporophytic

* Dipterocarpaceae and Turneraceae (Dicots)—Pollen cytology and cytokinesis not known.
† In these families the system of self-incompatibility has been studied.

have sporophytic incompatibility. This would suggest that the time of **S** allele action in heteromorphic species is premeiotic, and not meiotic, which may be the case in all homomorphic sporophytic species, and hence the mode of cytokinesis does not affect the incompatibility system.

SITE OF INHIBITION

The site of inhibition may be dependent on two phenomena: (1) time of **S** gene action producing precursors and (2) the place of conversion of precursors into incompatibility substances, occurring independently in the two sex organs. In the female organ, the **S** allele action may occur either (i) *after fertilization during embryogenesis;* (ii) *at the time of female sex determination in the flower;* or (iii) *before fertilization during or after megasporogenesis.* In (i) or (ii), which occur in most of the species so far studied, precursors of both the **S** alleles will be present during the whole (i) or most (ii) of the sporophytic generation. The conversion of the precursors into incompatibility substances will depend on the physiological conditions present in a particular part of the sex organ thus producing possibly five sites of inhibition, viz. (1) stigma, (2) style, (3) ovarian and ovular tissues, (4) pre- and (5) post-fertilization embryo-sacs.

In most self-incompatible species the conversion of the female precursors seems to occur in stigma or style or both, and thus inhibition occurs either on the stigma before germination or in the style at different stages of pollen tube growth. However, in a number of species the incompatibility is accompanied by no detectable slowing down of the growth of pollen tubes (Bateman, 1954; Brewbaker, 1957). In these plants conversion of the female precursors probably occurs in ovarian or ovular tissues or in pre- or post-fertilization embryo-sacs, but not in the style and stigma. In *Theobroma cacao* (Cope, 1958) it appears that the conversion of female precursors into incompatibility substances takes place in the unfertilized embryo-sac.

In the so-called gametophytic system only the male reaction is gametophytic, the female reaction being always sporophytic (showing the reaction of both the **S** alleles). A truly gametophytic species having both male and female reactions gametophytic would be possible if the **S** allele action occurred during or after megasporogenesis (iii). A species with such an incompatibility system would be only 50% self-sterile since only one pollen

tube at random enters each embryo-sac—a case indistinguishable in practice from a balanced lethal system found in some of the ring-forming *Oenotheras*. Such a system, though not yet found, is quite possible for it ensures survival of the species under certain adverse ecological conditions, as well as provides for the utilization of hybridity and its accompanying advantages.

In three species, *Brassica oleracea* (Sears, 1937), *Raphanus raphanistrum* (Kroh, 1956) and *Raphanus sativus* (Oelke, 1957), in which tests (grafting, maceration, amputation, etc. of the stigma) have been made, it has been found that the stylar tissues are devoid of incompatibility substances. It appears, therefore, that at least in these species the conversion of the female precursors into incompatibility substances does not occur in the stylar tissues and is localized in the stigma only. It is significant to note that in *Helianthus* (Compositae—Habura, 1957) which has sporophytic incompatibility and has trinucleate pollen grains, the inhibition occurs in the style and not at the stigma, as would be expected on the basis of the general relationship between pollen cytology and site of inhibition. This is presumably due to the conversion of the female precursors only in the style and not in the stigma in this species. Similarly in the trinucleate *Beta vulgaris* the inhibition occurs in the ovary presumably due to the lack of the conversion of female precursors both in the stigma and style. Thus, though the physiological relationship between pollen cytology and conversion of precursors fixes the site of pollen tube inhibition in relation to pollen cytology, the latter has no control over the precursor conversion in the female sex organ. This leads to some exceptions to the general rule of pollen-stigma inhibition in the sporophytic system of incompatibility. Similar exceptions are also possible to the general rule of pollen tube-style inhibition in the gametophytic system of incompatibility. This has been discovered in *Physalis ixocarpa* which has a gametophytic system (Pandey, 1957). In this species inhibition occurs somewhere in the ovary instead of in the style which is generally the case in this system (Pandey, unpub.).

Relative Significance of Factors Affecting the Evolution of two Types of Self-Incompatibility Systems

Schürhoff (1926) observed that, so far as systematics is concerned, the trinucleate condition of the pollen grains is related to previous evolutionary developments. The tendency for the pollen grains to have three nuclei is scattered unconnectedly among the separate branches of the phylogenetic tree. It is significant to note that a binucleate family is never found to be derived from a family with trinucleate grains. It is also observed that in the phylogenetic tree the trinucleate pollen grain families occur only in the terminal positions of the ramifications. These observations hold true even with the modern phylogenetic tree of Takhtajian (1954). The conclusion from the systematic and phylogenetic evidence is obvious. The binucleate condition of the pollen grain is a primitive character in angiosperms. The trinucleate pollen families have evolved only comparatively recently, independently and many times, from the binucleate pollen families. It may also be said that this developmental tendency is a continuous phenomenon and the trinucleate pollen species will probably continue to evolve in the future.

The suggestion that binucleate pollen species gave rise to trinucleate pollen species also leads to the conclusion that because of the physiological relationship between pollen cytology and the time of **S** gene action, the multi-allelic gametophytic system was the first incompatibility system evolved in the angiosperms. The multi-allelic sporophytic system with trinucleate pollen arose from the gametophytic species with binucleate pollen. The following consideration of this problem further supports this hypothesis.

(1) The evolution of the sporophytic system from the gametophytic system would involve precocious action of the **S** gene in the pollen mother cell. This may produce competition and dominance interactions in the pollen (Lewis, 1947). Since the competition interaction which may bring about self-compatibility is relatively rare in comparison to dominance interaction (Pandey, 1956*a*) and since the latter, because the style will still have individual action of **S** alleles, would be ineffective, a change from a gametophytic to a sporophytic incompatibility system could occur without involving the danger of complete breakdown of the self-incompatibility. Furthermore, there would be no requirement for the strict individual action of the **S** alleles in the style and this characteristic of the gametophytic system would be gradually lost, thus giving rise to a typical sporophytic system with dominance or individual action of the **S** alleles in the style as well as the pollen. This kind of evolution would also explain the occurrence of occasional different dominance relationships between the **S** alleles in the pollen and the style (Crowe, 1954). However, if the gametophytic system were to evolve from the sporophytic system, the dominance interaction in the style would bring about complete breakdown of self-incompatibility.

(2) In a gametophytic system a change from successive to simultaneous cytokinesis or from simultaneous to successive cytokinesis in the pollen mother cell would not affect self-incompatibility at all. However, in the multi-allelic sporophytic system which is possible only in species with simultaneous cytokinesis, a change to successive cytokinesis would break down the self-incompatibility because it would give rise to individual action of the **S** allele in the pollen and there would be dominance in the style. Cytokinesis being an easily variable phenomenon, many families having both kinds of cytokinesis in different genera, a primitive sporophytic system would have been very much exposed to easy breakdown of self-incompatibility.

The self-incompatible species which may be currently in the stage of evolution from a binucleate to a trinucleate pollen condition may produce plants which would be partly self-fertile due to the production of some trinucleate pollen which may be self-compatible due to competition interaction of **S** alleles. In the sporophytic species which may be currently evolving from simultaneous to successive cytokinesis in the pollen mother cell, some selfed seeds may be produced because of some pollen grains having an individual action of **S** alleles and the style having dominance interaction. About half the plants obtained from such seeds in both of the above cases would be homozygous for the **S** allele and would be self-incompatible and the other half would be heterozygous and may again produce some selfed seeds like their parent.

One of the most significant developments in the evolution of seed-bearing plants from their ancestors was the "telescoping" of the gametophytic generation with the consequent dominance of the sporophytes. In the gymnosperms the microgametophytic generation is reduced to a six nucleate tissue and this is further reduced to a three nucleate tissue in the angiosperms. Also the division in the microspore nuclei in these plants begins while the microgametophyte is still a part of the sporophyte. This progressive development towards maturing the microgametophyte before it is separated from the sporophyte has reached its culmination in many of the advanced families of the angiosperms. These families have trinucleate pollen in which both mitotic divisions have already occurred. In the evolution of the trinucleate microspores it is evident that selection must have favored an increase in the available metabolite content of the pollen mother cell; this would be essential to provide the energy for both the mitotic divisions to occur in the microspore. In many families this has been attained by the

development of tapetal periplasmodium. Since the increase in the metabolite content of the pollen mother cell brought about precocious **S** gene action and a chain of other physiological developments leading to the evolution of a sporophytic system of self-incompatibility, it suggests that the evolution of the sporophytic from the gametophytic system occurred as a corollary of the progressive evolution of the microgametophyte.

Since there is a causal relationship between the time of nuclear action (and pollen cytology in homomorphic species) and the type of incompatibility reaction, and since the former brings about the latter, it would be safe to assume that the second type of incompatibility evolved after the evolution of the two types of time of nuclear action (and pollen cytology), or concurrently with it, and could not have evolved before it.

In families in which the sporophytic type of self-incompatibility evolved as a result of the change in the time of nuclear action, the evolution took place in either of two ways: (1) The time of nuclear action was the dominant factor within the scope of which the genetic evolution of the second type of self-incompatibility occurred. This would be possible in the homomorphic species in which probably the change from the gametophytic to the sporophytic system did not involve a major breakdown of self-incompatibility. Or (2) the genetic evolution of self-incompatibility was the dominant factor which subordinated the physiology of the time of nuclear action by incorporating it as an integral part of its own **S** gene system. This was possible in the evolution of the heteromorphic system which presumably developed in the self-fertile species, the latter having arisen through the complete breakdown of self-incompatibility in their progenitors (Whitehouse, 1950).

As the second type of incompatibility system in the homomorphic species evolved within the limitation produced by the new time of nuclear action, the result was that the binucleate pollen families—because of normal (delayed) nuclear action—had a gametophytic system, trinucleate pollen families—because of precocious nuclear action—had a sporophytic system. Those families which have both binucleate and trinucleate species would have, therefore, both gametophytic and sporophytic systems, respectively, in the same family. The majority of homomorphic incompatible families, 45 out of 58 in which data are available (Schnarf, 1939; Brewbaker, 1957), have either binucleate or trinucleate species in one family (tables 1, 2 and 3). Of the ten families which are trinucleate, one (Gramineae) has successive cytokinesis in the pollen mother cells and, thus, can have only gametophytic incompatibility (table 2). The family Apocynaceae has both types of cytokinesis and, therefore, may have both systems of incompatibility depending on the nature of cytokinesis in the particular species. The other eight families, which have simultaneous cytokinesis, will have sporophytic incompatibility. All the 35 binucleate families (table 1) will have gametophytic incompatibility.

There are 13 homomorphic families known in which both types of pollen cytology are present (table 3). Of these Asclepiadaceae, Campanulaceae, and Araceae have successive and Liliaceae both types of cytokinesis in the pollen mother cells. Hence, the families Asclepiadaceae, Campanulaceae, and Araceae and the Liliaceous species which have successive division can have only gametophytic system. The other eight families and the Liliaceous species which are known to have only simultaneous cytokinesis (Schnarf, 1931; Sugiura, 1936, 1939, 1944) may have both gametophytic and sporophytic systems of self-incompatibility in the same family, depending on the nature of the pollen cytology in the individual species (fig. 7).

In the heteromorphic species, the evolution of secondary incompatibility (Whitehouse, 1950) built up a complex **S** gene which controls a number of morphological

as well as physiological characters related to the functioning of incompatibility. It is possible that in these species the time of nuclear action which is probably premeiotic (precocious) became an integral part of the **S** gene complex and is unaffected by the cytoplasmic constituents of the cell, which control this action in the homomorphic species. The physiologically important causal relationship between the time of nuclear action and the type of incompatibility would thus be maintained. They would all be sporophytic. This would also mean that in these species cytokinesis and pollen cytology would have no relationship with the time of nuclear action and the resultant independent system of incompatibility. This would explain the occurrence of binucleate as well as trinucleate pollen grains in heteromorphic species which have a sporophytic reaction and should normally have only trinucleate grains (table 4). Since pollen cytology in the heteromorphic species, though physiologically unrelated to the time of nuclear action, would still govern the conversion of precursors into incompatibility substances, the physiological relationship between pollen cytology and site of inhibition remains intact. Thus binucleate pollen is inhibited during pollen tube growth and trinucleate pollen during germination (fig. 3B). The relationship between the incompatibility system and the site of inhibition may, therefore, be disturbed by the variation in the site of precursor conversion in the male as well as female organs.

Summary

1. A hypothesis has been developed to explain the relationship between the following factors and the evolution of gametophytic and sporophytic systems of self-incompatibility: (i) pollen cytology; (ii) time of nuclear action; (iii) production of pollen growth substance; (iv) cytokinesis in the pollen mother cell and (v) site of inhibition.

2. It is suggested that the time of **S** gene action in the heteromorphic species is premeiotic whereas in the homomorphic species it is meiotic (sporophytic) or post-meiotic (gametophytic).

3. It has been proposed that the selection for a critical amount of available metabolites in the microspore mother cell may have been one of the basic physiological mechanisms in the evolution of self-incompatibility systems. A slight excess of metabolite in the pollen mother cell would trigger a precocious **S** gene action and a chain of other reactions associated with sporophytic system of incompatibility. A critically lower concentration of metabolites would trigger a delayed **S** gene action and an alternative chain of events associated with gametophytic incompatibility.

4. The available data suggest that the qualitative characteristics of the physiological mechanism of the expression of incompatibility reaction are under the control of the **S** gene complex, while the time of the actual triggering of the physiological chain reaction and its extent of development are controlled by certain cytoplasmic constituents of the species.

5. According to the above hypothesis the gametophytic and sporophytic systems of self-incompatibility may be found in the same family which has simultaneous cytokinesis and has both kinds of species having either binucleate or trinucleate pollen grains.

6. From the systematic and phylogenetic evidence it has been concluded that the homomorphic sporophytic species with trinucleate pollen evolved from the gametophytic species with binucleate pollen. The genetic implications, which support this hypothesis, have been discussed.

7. It is suggested that the evolution of the sporophytic system of self-incompatibility from the gametophytic system occured as a result of the progressive evolution of the microgametophyte.

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INTERACTION BETWEEN INVERSION POLYMORPHISMS OF TWO CHROMOSOME PAIRS IN THE GRASSHOPPER, *MORABA SCURRA*.

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INTRODUCTION

In studying the evolutionary dynamics of polymorphisms in natural populations, most of the work in the past has concentrated on single genetic entities. The Work of Dobzhansky and his collaborators on the inversion sequences in the third chromosome of *Drosophila pseudoobscura*, the studies of Lamotte (1951) on shell color and banding in the snail *Cepaea nemoralis*, and those of Allison (1955) on abnormal hemoglobins in man are examples of genetic studies of polymorphisms controlled by alleles at a single locus, or else by blocks of genes which act in heredity as single loci.

A new order of complexity is introduced when an attempt is made to study *simultaneously* two or more segregating systems in the same population because of the possibility, indeed the probability, that the evolutionary fates of the different polymorphic systems are not independent. Such will be the case if there is interaction between the different loci in the determination of the fitness of various genotypes. For example, the fitness of individuals of the genetic constitution AA may be greater than those with the constitution aa if at a second locus these individuals are, let us say, BB; but this fitness relationship may be reversed in the presence of bb at the second locus. In such cases the polymorphisms A,a and B,b are not independent in their fates in the population. Levitan (1955, 1958) in his studies of the inversion polymorphisms of *Drosophila robusta* has evidence of a small interaction of this sort and Cain and Sheppard (1954a, 1954b) have found such interactions for shell color and banding in *Cepaea nemoralis*.

One of the most favorable materials for the study of these interactions and their evolutionary consequences is the Australian grasshopper, *Moraba scurra*. In an earlier paper (White, 1957) evidence was presented that the pericentric inversions, carried on two different chromosome pairs in this species, are not combined at random in the adult male individuals of certain natural populations. The deviations from random combination were regarded as proof of a genetic interaction between the two systems of cytological polymorphism, so far as their effects on viability are concerned. The significance of this interaction effect has been discussed and a hypothesis as to how it could have arisen has been suggested (White, 1958).

The present paper has two purposes. First we shall present added data on the interaction effect from recent collections. The data published previously relate to six adult male samples collected in 1955 and 1956 at various localities in South Eastern Australia. Further data on two of these populations (at Wombat, New South Wales, and Royalla "B," Australian Capital Territory) were obtained in 1958 and 1959. Statistical analysis of the more recent data provides

additional evidence for the interaction effect, which is thus shown to be no mere "flash in the pan," but a consistent phenomenon, occurring in at any rate most of the populations in which both chromosome pairs are polymorphic, and manifesting itself regularly each year.

Second we shall present a method for dealing with observations that is designed to provide a better understanding of the past and future evolution of such complex polymorphic systems. Specifically we shall attempt to answer two questions. Is the present genetic composition of the populations what one would expect from the estimates of fitness of the various genotypes? What is likely to be the future genetic history of these populations?

The 1958 Collections

The chromosome frequencies given in table 1 were calculated from adult male samples collected during the winter months. The results of our analyses carried out in 1958 are presented in tables 2 and 3. In these populations there are two sequences of the "CD" chromosome, *Standard* and *Blundell* and two of the "EF" chromosome, *Standard* and *Tidbinbilla*. The Standard chromosomes are both metacentric, Blundell and Tidbinbilla being acrocentric elements. For information on the life cycle of *Moraba scurra*, location of the colonies studied, appearance of the chromosomal inversions and other details, reference should be made to the earlier papers (White 1956, 1957, White and Chinnick, 1957).

Table 1. *Chromosome frequencies in 1956, 1958, and 1959*

		1956	1958	1959
Wombat:	Blundell CD	0.84800	0.85246	0.87500
	Tidbinbilla EF	0.14950	0.13866	0.13955
Royalla "B":	Blundell CD	0.66667	0.67267	
	Tidbinbilla EF	0.20333	0.22823	

	χ^2	d.f.	P
Wombat CD	4.61	2	≃.1
Wombat EF	1.01	2	≃.6
Royalla CD	0.07	1	≃.8
Royalla EF	1.59	1	≃.2

Table 2. *Composition of the population at Royalla "B," 1958, with deviations from proportionality to marginal frequencies*

		Chromosome CD			
		St/St	St/Bl	Bl/Bl	
Chromosome EF	St/St	22 (+4.61)	96 (+4.43)	75 (−9.04)	193
	St/Td	8 (−3.53)	56 (−4.73)	64 (+8.26)	128
	Td/Td	0 (−1.08)	6 (+0.31)	6 (+0.77)	12
		30	158	145	333

$\chi^2_{(4)} = 6.29$, P ≃ 0.18.

It will be seen that no significant changes have occurred in the chromosome frequencies of either the Wombat or the Royalla "B" population from 1956 to 1959 (table 1), since none of the chi squares approaches statistical significance. Thus, for some purposes it might be legitimate to simply add together our samples of 1956, 1958 and 1959. However, in tables 2, 3 and 4, the data of the later years are presented separately, for comparison with those previously published.

We test for the interaction effect by

Table 3. *Composition of the population at Wombat, 1958, with deviations from proportionality to marginal frequencies*

		Chromosome CD			
		St/St	St/Bl	Bl/Bl	
Chromosome EF	St/St	16 (+4.28)	141 (+6.27)	379 (−10.55)	536
	St/Td	0 (−4.13)	42 (−5.51)	147 (+9.64)	189
	Td/Td	0 (−0.15)	1 (−0.76)	6 (+0.91)	7
		16	184	532	732

$\chi^2_{(4)} = 8.227$, P ≃ 0.09.

TABLE 4. *Composition of the population at Wombat, 1959, with deviations from proportionality of marginal frequencies*

Chromosome EF		Chromosome CD			
		St/St	St/Bl	Bl/Bl	
	St/St	7 (−0.38)	100 (+7.01)	324 (−6.63)	431
	St/Td	3 (+0.55)	22 (−8.85)	118 (+8.30)	143
	Td/Td	0 (−0.17)	4 (+1.84)	6 (−1.67)	10
		10	126	448	584

$\chi^2_{(4)} = 5.949$, P = .20.

determining the deviations from proportionality to the marginal frequencies, in the case of each of the nine genotypes. These deviations were found to be similar to those encountered previously (e.g., the Bl/Bl, St/St genotype is always deficient, the St/Bl, St/St and Bl/Bl, St/Td genotypes are present in excess, etc.). It should be pointed out that deviations calculated in this manner cannot be due to heterosis, although heterosis is undoubtedly present.

As with the earlier data the deviations of the two samples, considered separately, are not statistically significant, as shown by chi square tests. What we need, clearly, is a statistical test which can be applied to the data as a whole, including samples collected at different localities and in different years, and which show different chromosome frequencies. In the earlier paper (White, 1957) a statistical analysis by Dr. B. Griffing was presented which involved the partitioning of the $\chi^2_{(4)}$ into four orthogonal components. This procedure established that an interaction between the two systems of cytological polymorphism does, in fact, exist. However, since the analysis is of a relatively sophisticated type, a simpler test of significance may be preferable. The results of such a test (suggested by Professor J. B. S. Haldane *in litt.*), applied to the six samples considered in the earlier paper as well as to the samples studied in 1958 and 1959, are given in table 5. Each 3 × 3 contingency table is condensed to a 2 × 2 table by adding the rarer homozygous classes to the heterozygotes $\left(\text{e.g. for Wombat, 1958, we have } \frac{157}{43}\middle|\frac{379}{153}\right)$. We then calculate a $\chi^2_{(1)}$ for each 2 × 2 table. Values of χ are then calculated; those for samples with negative deviations in the top right and lower left cells are regarded as positive, those with negative deviations in the other two cells as negative (in the case of the 9 samples in table 5 all χ's are positive). The statistic $t = \frac{\sum\chi}{\sqrt{n}}$ is used as a normal deviate. The result of this test is to establish the reality of the interaction effect with virtual certainty ($p \simeq 0.00001$).

If we assume that panmixia occurs in these populations, the relative viabilities of the nine genotypes in each of the samples may legitimately be estimated by the ratio of the number of individuals actually found to those expected on the binomial square rule. The fact that no significant changes in the chromosome frequencies are taking place from generation to generation, in these populations, renders the objections of Wallace (1958) and Novitski and Dempster (1958) to

TABLE 5. *Test of significance of the interaction effect*

		$\chi^2_{(1)}$	χ
Wombat	1956	0.81887	0.90491
Wombat	1958	3.90552	1.97624
Wombat	1959	2.17920	1.47621
Hall	1955	1.29005	1.13580
Hall	1956	4.22536	2.05557
Royalla "A"	1955	2.81785	1.67865
Royalla "B"	1956	0.75647	0.86975
Royalla "B"	1958	4.09607	2.02387
Williamsdale	1956	2.88523	1.69860
			13.81960

$\frac{\Sigma(\chi)}{\sqrt{N}} = \frac{13.81960}{3} = 4.607$, $P \simeq 0.00001$.

TABLE 6. *Estimated relative viabilities of the 9 genotypes in 1956, 1958 and 1959, calculated from the deviations from expectation (on the basis of the Hardy Weinberg ratios) by the method of Haldane (1956)*

St/Bl; St/St arbitrarily taken as 1.000 in each sample.

		Chromosome EF	Chromosome CD		
			St/St	St/Bl	Bl/Bl
Wombat	1956	St/St	0.789	1.000	0.922
Wombat	1958	St/St	1.353	1.000	0.924
Wombat	1959	St/St	0.970	1.000	0.917
Royalla	1956	St/St	0.791	1.000	0.834
Royalla	1958	St/St	0.932	1.000	0.752
Wombat	1956	St/Td	0.801	0.876	1.004
Wombat	1958	St/Td	0.000	0.919	1.113
Wombat	1959	St/Td	1.282	0.672	1.029
Royalla	1956	St/Td	0.670	1.006	0.901
Royalla	1958	St/Td	0.573	0.976	1.086
Wombat	1956	Td/Td	0.000	1.308	0.645
Wombat	1958	Td/Td	0.000	0.272	0.564
Wombat	1959	Td/Td	0.000	1.506	0.645
Royalla	1956	Td/Td	0.657	0.657	1.067
Royalla	1958	Td/Td	0.000	0.707	0.688

such a procedure inapplicable in the present instance. The relative viabilities for the 1958 and 1959 samples, calculated by the method of Haldane (1956), which includes a correction to remove bias, are given in table 6, which may be compared with table 8 of White, 1957. The St/Bl; St/St genotype is used as a standard (viability = 1.000) in the case of each sample.

Such calculations suffer from one serious weakness, namely the very considerable sampling error in the case of five of the nine genotypes, whose numbers are in all cases very small. Thus it is only the relative viabilities enclosed within the dotted line in table 6 that should be regarded as at all reliable. It will be noted that, in the case of the values within the dotted line, there is good agreement between those for 1956, 1958 and 1959, and that this agreement is especially close for the most numerous Bl/Bl; St/St genotype. In view of this, and because the chromosome frequencies of these populations have not changed from 1956 to 1959, we have thought it legitimate to pool the data for the three years (table 7). It seems likely that the relative viabilities in table 7 are more reliable estimates, in the case of the rarer genotypes, than those in table 6. But it would obviously be necessary to study samples ten to a hundred times larger in order to obtain any close accuracy in the case of such rare genotypes as St/St; Td/Td. We feel that any differences in the viability constants which may occur from one year to another in the same locality are likely to be trivial compared to the inevitable inaccuracy of the method by which they are determined. Differences between the chromosome frequencies from one locality to another, however, clearly imply that the relative viabilities of the genotypes are not the same throughout the distribution area of the species, and may vary over a distance of a few miles. The Royalla A and B localities are about a mile apart and both are situated on a strip of ungrazed land along a railroad. These populations were no doubt formerly continuous and are probably not completely cut off from one another even now. There is no significant difference between the frequencies of the CD sequences at these localities ($\chi^2_{(1)} = 0.21$), but a real difference may exist in the frequencies of the EF sequences ($\chi^2_{(1)} = 3.5495$). It would hence be dangerous to pool the data for Royalla A and Royalla B in order to estimate the relative viabilities of the nine genotypes.

THE ADAPTIVE SURFACES

If estimates are available of the fitnesses or adaptive values of the various

TABLE 7. *Estimated relative viabilities, calculated in the same manner as in table 6, but with the data of 1956, 1958, and 1959 combined*

	Chromosome EF	Chromosome CD		
		St/St	St/Bl	Bl/Bl
Wombat	St/St	1.002	1.000	0.927
Royalla	St/St	0.842	1.000	0.808
Wombat	St/Td	0.646	0.849	1.044
Royalla	St/Td	0.636	0.997	0.974
Wombat	Td/Td	0.000	1.054	0.626
Royalla	Td/Td	0.393	0.682	0.916

genotypes in a population, it is possible to predict what the eventual genotypic composition of the population will be and at what rate gene frequencies should change, given that the population has a certain composition at any particular time. Particularly, one would like to know whether the population will reach a stable polymorphic condition or whether it will tend to monomorphism. A general theoretical treatment of evolution in multi-locus systems is not appropriate here and will be dealt with in a separate paper. There is enough in the existing literature, however, to provide a basis for the present analysis. Rigorous demonstrations and original expositions of the concepts to be used here may be found in the papers of Wright (1949, 1956), Li (1955), Kimura (1956), and Lewontin (1958).

In a polymorphic system of two loci with two alleles at each locus, there will be nine genotypes: AABB, AABb, AAbb, AaBB, etc. To each genotype is assigned a weight, W, which is the fitness or adaptive value of that genotype. Denoting the frequency of the ith genotype by Z_i and the fitness by W_i, the mean adaptive value of the population, $\bar{W}$, is defined as

$$\bar{W} = \sum Z_i W_i.$$

Mean adaptive value, so defined, will be different for different genotypic compositions of the population; it will be higher when genotypes with larger values of W_i predominate and lower when less fit genotypes are in greatest frequency. In a random mating population the frequency of any genotype at one locus is given by the binomial square rule and if we ignore the effects of linkage between the loci, the frequency of genotypes for the loci simultaneously will be the appropriate term in the product of the two binomial expansions of the separate loci. Thus, if the frequency of A is q_1 and that of B is q_2, the genotype frequencies at the A locus are: q_1^2 AA, $2q_1(1 - q_1)$ Aa, and $(1 - q_1)^2$ aa, while for the B locus the frequencies are: q_2^2 BB, $2q_2(1 - q_2)$ Bb and $(1 - q_2)^2$ bb. For the combined genotypes the frequencies will be:

$$\begin{array}{cc} \text{AABB} & \text{AaBB} \quad \cdots \text{aabb} \\ q_1^2 q_2^2 & 2q_1(1-q_1)q_2^2 \cdots (1-q_1)^2(1-q_2)^2 \end{array} \qquad (1)$$

Although it is often assumed that there is no complication due to linkage when the genes are on different chromosomes, this is incorrect and the frequencies given above are only approximate even for cases of free recombination. Because the effects of linkage can be shown to be small, however, we will ignore them in the discussion.

Using the distribution of genotypes given in (1) it is then possible to calculate $\bar{W}$ for any combination of gene frequencies q_1 and q_2 and this may be put in the form of a surface or topography. The two horizontal dimensions represent the frequencies q_1 and q_2 and the vertical height at each point represents the mean fitness, $\bar{W}$. Thus, a sort of "adaptive landscape" is formed with peaks, valleys, slopes, and saddles. At any particular time the population may be represented as a point on the q_1, q_2 plane and a change in q_1 and q_2, that is the movement of the point, will be governed by the conformation of the topography. If the fitnesses of the various genotypes are constants and do not depend upon the frequencies of the genotypes, it can be demonstrated that the gene frequencies will change in such a way as to increase the value of $\bar{W}$. That is, the population will tend to "ascend" the slopes of the topography, coming to rest on a "peak." If there are several adaptive peaks in the landscape, the one to which the population moves will be governed by the present position of the population and by the slope of the landscape in the neighborhood of that position. Thus, the population may easily reach an equilibrium genetic composition which does not correspond to the maximum possible $\bar{W}$ over the whole landscape, but may come to rest on a rela-

tively low peak. This is another way of saying that natural selection is opportunistic and that evolution does not always result in the "best of all possible worlds."

In figures 1–10 we give the topographies calculated for each of the 8 samples, and for the pooled data of 1956 and 1958 in the case of Royalla "B" and Wombat populations. The method of computing these topographies was as follows. The frequencies of Blundell CD chromosomes (q_1) and of Tidbinbilla EF chromosomes (q_2) were varied between 0 and 1 at intervals of .05. The 21 values of q_1 (.00, .05, .10$\cdots$1.00) and the 21 values of q_2 in all possible combinations give 441 points on the q_1q_2 plane. For each combination of q_1 and q_2 the frequencies of the 9 genotypes were computed according to expression (1). These frequencies, Z_i, were then multiplied by the appropriate value of W_i taken from table 6 and summed to give $\bar{W}$. A value of $\bar{W}$ thus resulted for each of the 441 possible points on the q_1q_2 plane. By interpolation between points it was then possible to draw the continuous topographic lines connecting points of equal mean adaptive value.[1] The black dot on each topography represents the actual composition of the population and the arrow indicates the direction in which that composition should change in future generations if the topography truly represents the mean adaptive values at each of the 441 points on each surface.

All the topographies have certain features in common. They show a kind of ridge running obliquely across the surface from some point on the upper side of the square to a point on the right hand side. On either side of the ridge, but especially in the direction of the lower left hand corner, the surface falls away steeply. In the case of those samples in which no St/St; Td/Td individuals were encountered the mean viability at this corner of the topography appears as zero.

The two ends of the ridge are higher than the middle, so that there is a kind of saddle. Moreover, in every instance the actual population is located on this saddle. This is especially clear for the Royalla samples, Hall, 1956, and Williamsdale. The topographies for Wombat, 1956, and Hall, 1955, look somewhat different from the others but Wombat, 1958, and the topography for the Wombat data of 1956 and 1958 look fairly similar to the "typical" topographies. In figure 11 we give a diagrammatic representation of a typical topography, showing how each of the four corners corresponds to a cytologically monomorphic population. We may speak of the ridge as orientated in the AB direction or axis, the axis which crosses the saddle perpendicular to the ridge being referred to as the CD axis.

The general similarity of the topographies which we have calculated is extremely striking, when we consider how remote the relationship between the different colonies must be. The Wombat population belongs to the 17-chromosome race, and the others to the 15-chromosome race, so that to find a common ancestor of the Wombat population and the remaining colonies it might be necessary to go back several million years (i.e., generations). The two Royalla colonies ("A" and "B") are situated only about a mile apart and hence must be regarded as closely related. These localities are about six miles from Williamsdale and about 30 miles (in the opposite direction) from Hall. The vagility of this wingless insect is so low that to find a common ancestor of the Hall and Royalla populations it would probably be necessary to go back at least 3,000 years.

Such topographies obviously include considerable sampling errors, as previously pointed out. Such sampling errors, however, are almost certainly not large enough to obscure the general

[1] These lines might be called "isodapts."

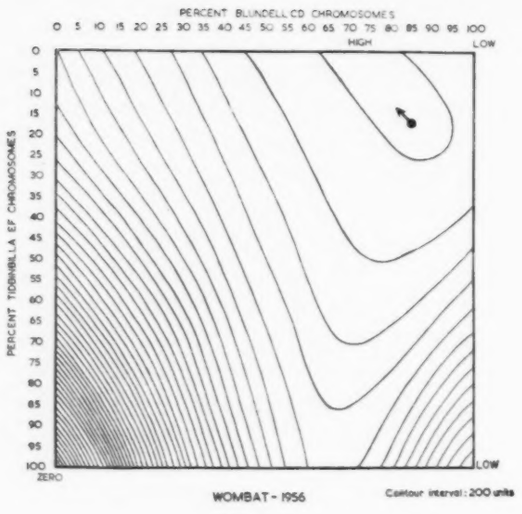

FIG. 1

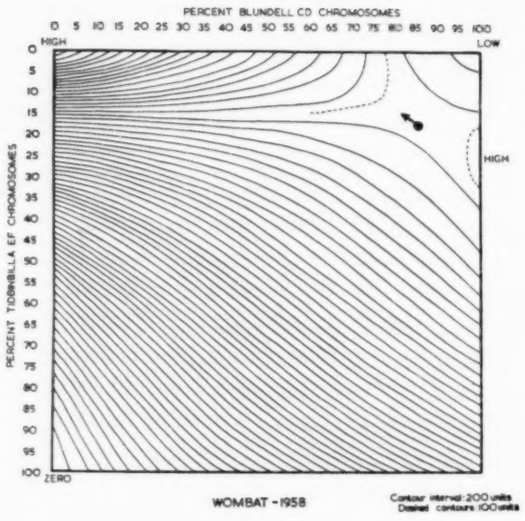

FIG. 2

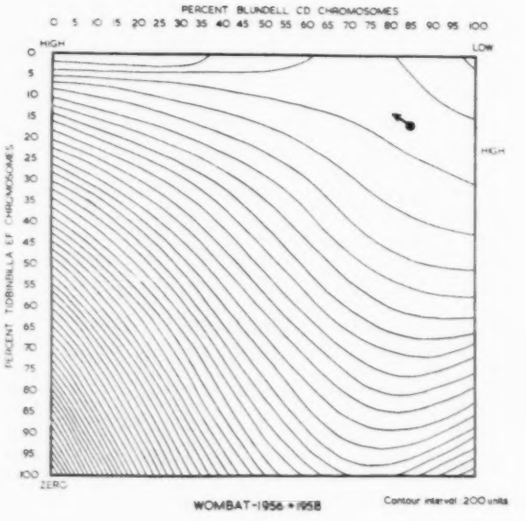

FIG. 3

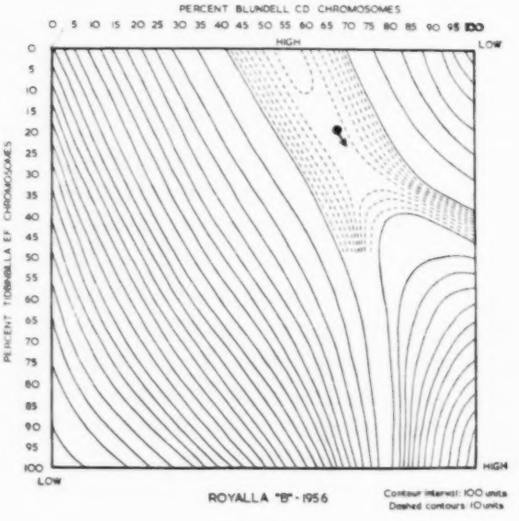

FIG. 4

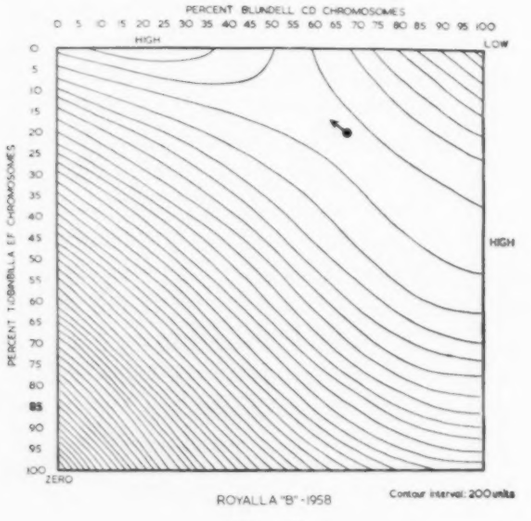

FIG. 5

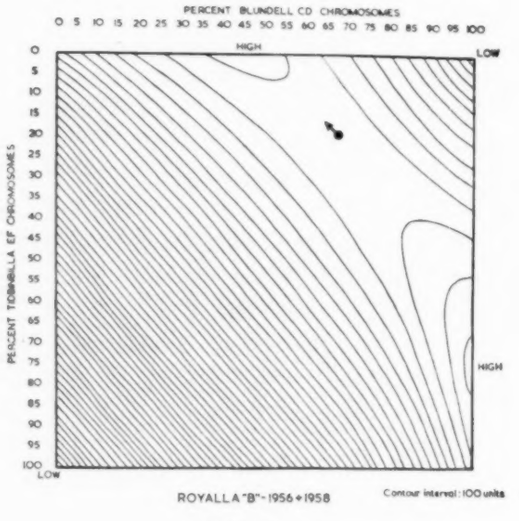

FIG. 6

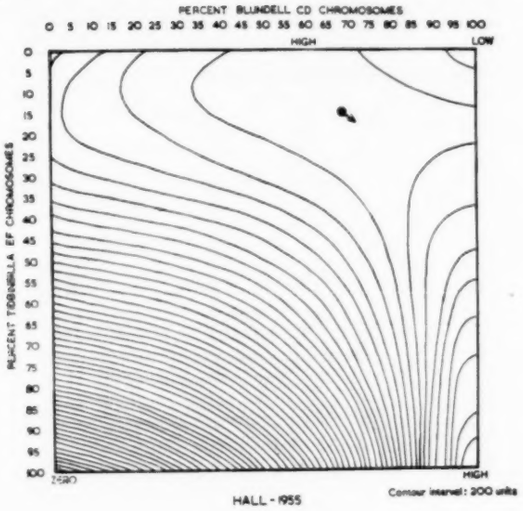

FIG. 7

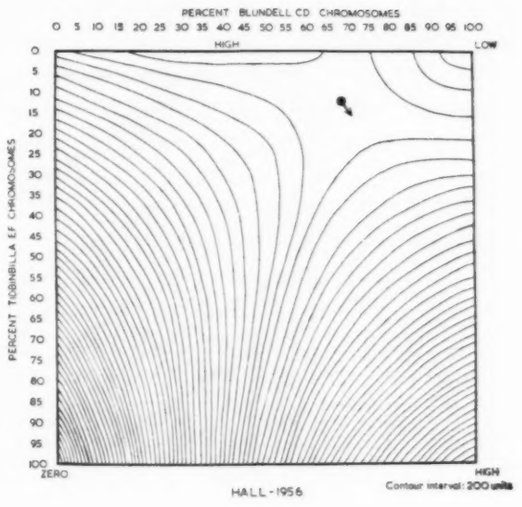

FIG. 8

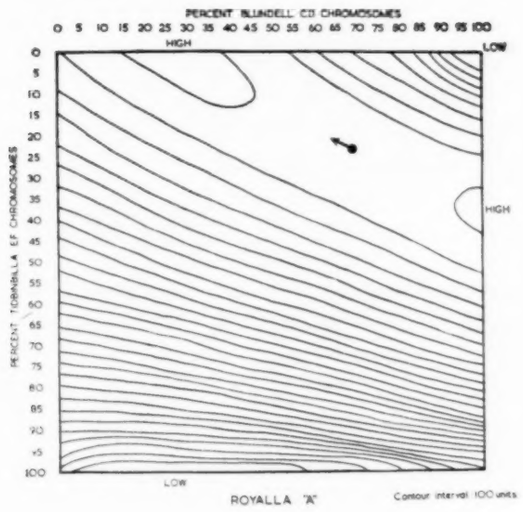

FIG. 9

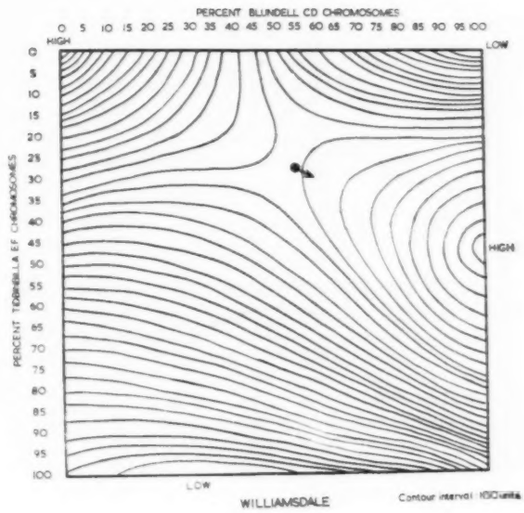

FIG. 10

FIGS. 1–10. The topographies (explanation in text).

features of the adaptive landscape. That this is so is demonstrated by comparing the topographies calculated from different years in the same locality. If one takes the extreme position that no real change in adaptive values takes place from year to year, a position that is hardly credible considering the lack of constancy of physical factors of the environment, then the differences between topographies in two different years from the same locality can be regarded as due entirely to sampling error. While the different years certainly do not produce identical topographies, the general features are the same. The location of the saddle point remains virtually unchanged from year to year and the general orientation of the ridge remains "northwest" to "southeast." The main change from year to year is a shift in the angle of the ridge with a resultant shift of the peak positions along the margin. This phenomenon is shown for Wombat (figs. 1 and 2) and Royalla "B" (figs. 4 and 5). The combined topographies (fig. 3 for Wombat and fig. 6 for Royalla) can be regarded as the mean surfaces for the two years and are intermediate between them. The two samples of the Hall

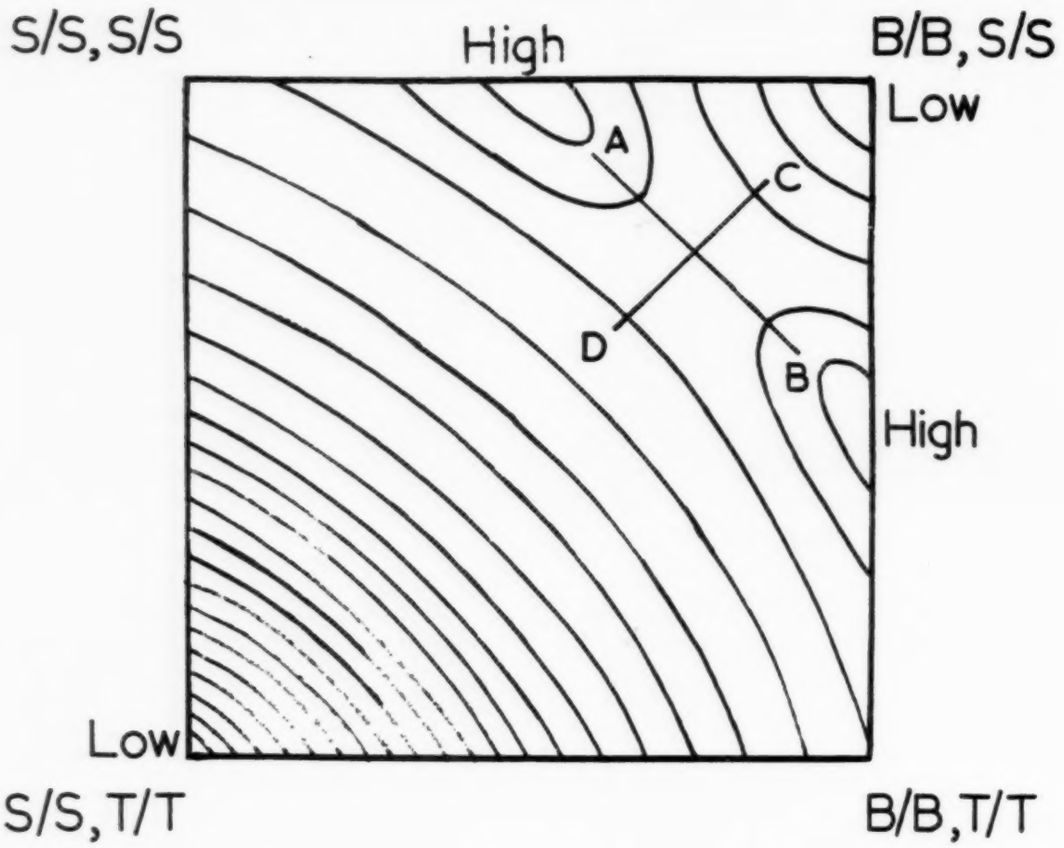

FIG. 11. Diagrammatic representation of a typical topography. The constitution of the monomorphic populations at the four corners is indicated. AB and CD are the two axes discussed in the text.

population (figs. 7 and 8) show practically no change.

THE GENETIC EQUILIBRIUM OF THE POPULATIONS

The topographies refer solely to one component of the selective value, namely male viability up to the adult stage. They take no account of other components such as female viability, sexual activity and fecundity in both sexes; all of which must be involved in the determination of the genetic equilibrium. Moreover, they are based on the assumption that panmixia obtains, an assumption which may not be strictly true (see White, 1957, p. 326). In spite of these reservations we believe it is worth while to discuss the genetic equilibrium which appears to obtain in these populations in the light of the evidence provided by the topographies. It is at any rate evident that in all the localities where these samples were collected a population consisting mainly of St/St, Td/Td individuals would be severely handicapped, whatever the fecundity of that genotype.

Stability in the direction of the CD axis seems assured for all populations, i.e., there is little likelihood of them "slipping down off the ridge." Equilibrium along the AB axis is less certain. At first sight it would seem that each population should in future generations "climb" from its present position on the topography up whichever slope is steeper until it reaches the highest point. The apparent final equilibrium state of each sample is shown in table 8. It should be pointed out that the surfaces in the immediate vicinity of the dots that indi-

TABLE 8. *Apparent final equilibrium state of the samples*

Hall	1955 & 1956	Bl/Bl; Td/Td
Wombat	1956	St/Bl; St/St
Wombat	1958	St/St; St/St
Wombat	1956 + 1958	St/St; St/St
Royalla A	1955	St/Bl; St/St
Royalla B	1956	Bl/Bl; Td/Td
Royalla B	1958	St/Bl; St/St
Royalla B	1956 + 1958	St/Bl; St/St
Williamsdale	1956	Bl/Bl; St/Td

cate the existing composition of the samples are in several instances so nearly horizontal that a relatively minute change in the slope would determine whether the population should move along the AB axis towards A or towards B.

The following hypotheses concerning these populations may be considered:

I. There is no true equilibrium and the populations will in actuality climb from their present position towards either A or B.

II. An equilibrium exists.

IIa. An equilibrium exists because the actual selective values are different from the relative viability constants, i.e., the various components of the selective value are imperfectly correlated. For example, the high viability of some genotypes may be accompanied by low fecundity.

IIb. An equilibrium exists, because the selective values of some or all of the genotypes are frequency-dependent. In particular, the selective values of some genotypes may decline as these genotypes become more frequent in the population.

IIc. A pseudo-equilibrium exists, the direction of the slope along the AB axis in the immediate vicinity of the existing constitution undergoing a periodic reversal every few generations (see fig. 12 in which it is assumed that such a reversal is related to "wet" and "dry" years).

Hypothesis I states essentially that the present composition of the populations is an historical relic, the populations being still in a state of directed gene frequency change. Agaist such a hypothesis is the fact that each population is at *its own saddle point.* The loca-

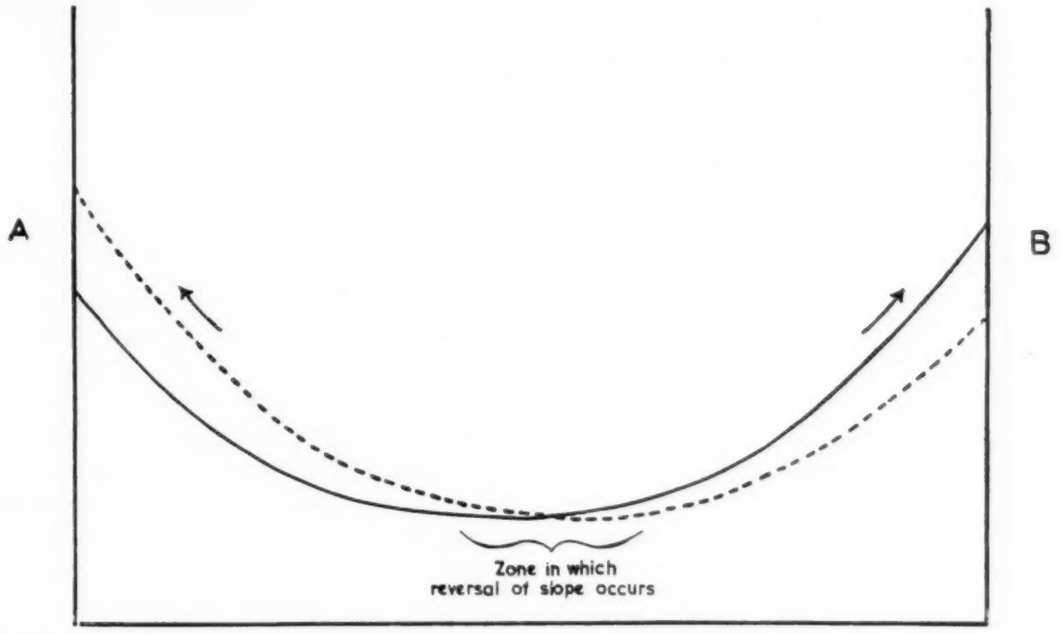

FIG. 12. Diagram illustrating hypothesis II c. The figure represents a section of a topography along the AB axis. The full line is supposed to indicate the slope in a wet year, the dotted line is the slope in a dry year. The zone in which reversal of the slope occurs is indicated.

tion of the saddle for each population is slightly different as is the location of the point representing the actual gene frequencies. That each population should have reached its own saddle but not begun to climb the ridge is difficult to accept especially if these populations are tens of thousands of generations old, as we believe them to be. This objection is somewhat mitigated by the extreme flatness of the adaptive surface around the saddle point so that predicted changes in gene frequency would be of the order of .0002 to .0003 per generation. While this slow rate of change might account for a stalling of the evolutionary process near the saddle point, it would not in itself explain why each population should pass near the saddle point on its way to one or the other of the peaks.

Some insight into this problem can be gained by calculating the theoretical path of a population along the gene frequency plane under the influence of the estimated gradients in mean adaptive value. This can be done by regarding a system of two segregating loci with two alleles at each locus as if it were a single locus with four alleles. These alleles, which are the four gametic types, are St;St, St;Td, Bl;St, and Bl;Td. Assigning the symbols x, y, z, and u to the frequencies of these four gametic phases respectively, the change per unit time in the frequency of the alleles can be represented by four differential equations:

$$\frac{dx}{dt} = x[W_x - \bar{W}] - rW_H(xu - yz) \quad (2)$$

$$\frac{dy}{dt} = y[W_y - \bar{W}] + rW_H(xu - yz) \quad (3)$$

$$\frac{dz}{dt} = z[W_z - \bar{W}] + rW_H(xu - yz) \quad (4)$$

$$\frac{du}{dt} = u[W_u - \bar{W}] - rW_H(xu - yz) \quad (5)$$

where W_x, W_y, W_z and W_u are the average fitnesses of the phases St;St, St;Td, Bl;St, and Bl;Td in all their combinations, $\bar{W}$ is the average adaptive value of the population at the given combination of gene frequencies, r is the proportion of recombination between the two loci, which for our case is .50 since we are dealing with two different chromosomes, and W_H is the fitness of the double heterozygote St/Bl;St/Td. One method of deriving these equations can be found in Kimura, 1956. The variable of time can be eliminated from these equations and the set of four dependent equations reduced to three independent equations by dividing (2), (3), and (4) by (5). The simultaneous solution of the three new equations $\frac{dx}{du}$, $\frac{dy}{du}$ and $\frac{dz}{du}$ will give the path of gametic frequency change of a population starting with some initial composition x_0, y_0, z_0 and u_0. Finally the gametic frequencies can be converted into gene frequencies by noting that

$$(u + z) = \text{frequency of Bl},$$
$$(u + y) = \text{frequency of Td}.$$

Such equations are not easily soluble by analytic methods so that we have employed numerical solutions using the Runge-Kutta and Milne methods adapted to the IBM 650 computer.

The results of the solution of these equations are shown in figure 13 for the Royalla "B" sample of 1956. Given in the figure is the topographic map of the adaptive surface as shown in figure 1, upon which has been superimposed the calculated trajectories for populations starting with different initial gene frequencies.

Two points emerge from this analysis. First, the population may go to either of the two adaptive peaks, depending upon the starting point of the population. Second, and more interesting, is the fact that the population generally does not pass directly to the peak from a low point, but goes to the peak *by way of the saddle*. This is true, at least, for populations whose initial composition is either St;Td, or Bl;St.

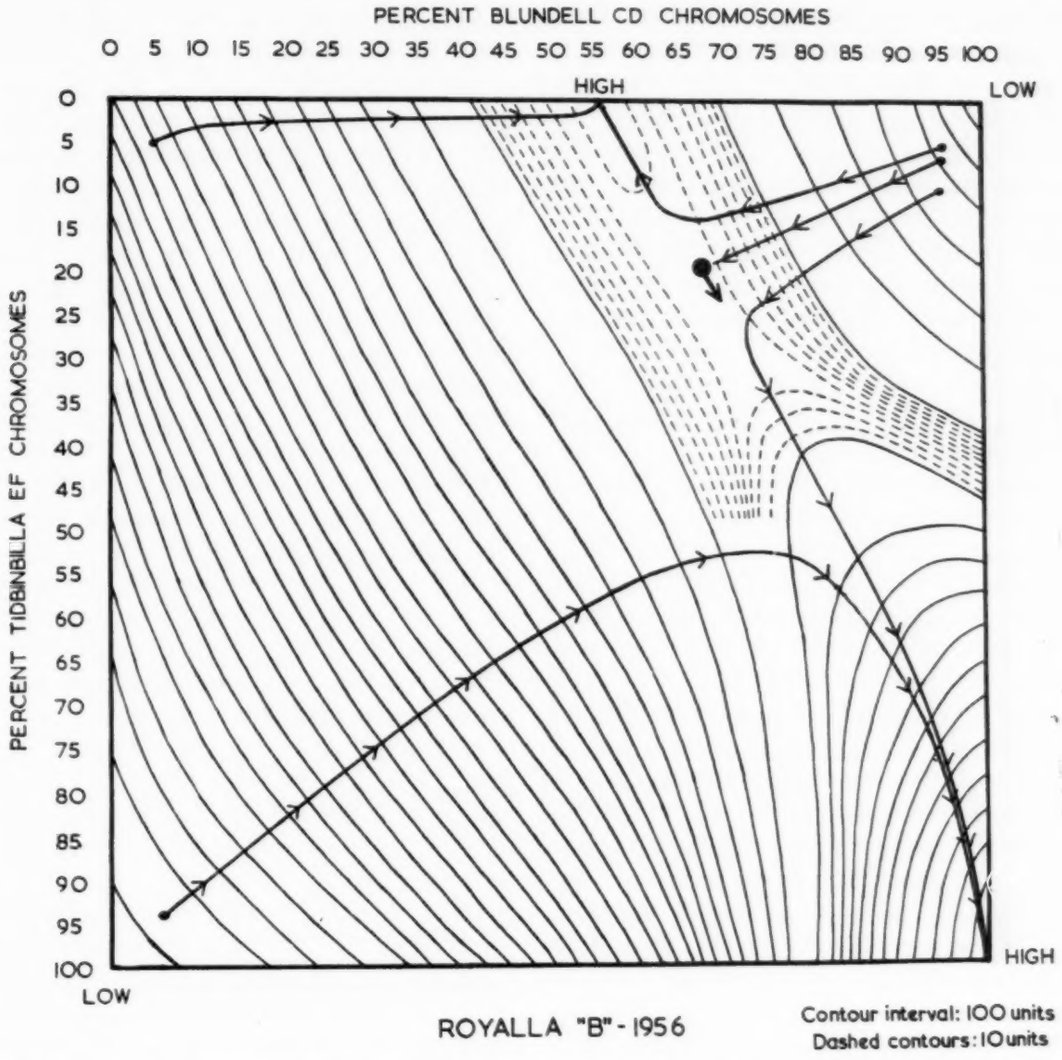
PERCENT BLUNDELL CD CHROMOSOMES
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
HIGH
LOW
PERCENT TIDBINBILLA EF CHROMOSOMES
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
LOW
HIGH
ROYALLA "B" - 1956
Contour interval: 100 units
Dashed contours: 10 units

FIG. 13. The theoretical trajectories of gene frequency change in the Royalla B population, superimposed onto the adaptive topography for that surface. Arrows indicate the direction of the trajectories in time. Data of 1956.

Thus we might imagine, if hypothesis I were the correct one, that the present location of the populations near their saddle points is an historical relic due to the very slow rate of gene frequency change near the saddle and to the initial composition of the populations either at the time the inversions arose or at the time of establishment of the colonies.

We do not favor this hypothesis because of the high degree of coincidence that seems necessary to it and because the rate of gene frequency change, slow as it is, would result in considerable changes over a period of 500 to 1,000 generations. Nevertheless we cannot completely reject this hypothesis, unlikely as it may seem, since all evolutionary changes seem unlikely in retrospect.

A formal decision among hypotheses IIa, IIb, and IIc does not seem possible but experimental verification of one or a combination of them is possible. These hypotheses are not incompatible with one another and all three may be true to some extent.

Hypothesis IIa seems to imply that genetic co-adaptation of the inversion sequences has proceeded differently in

the case of the genes affecting the various components of fitness. What data exist from Drosophila suggest that the opposite is true and that viability, fecundity, and longevity are positively, rather than negatively correlated (see especially Vetukhiv, 1954, 1956, 1957, and Wallace, 1948). This hypothesis is then rather unattractive but without experimental disproof cannot be rejected out of hand.

Hypotheses IIb and IIc seem much more plausible. If we assume that the various genotypes are to some extent occupying various different niches within the general environment, then as a particular genotype becomes more abundant it will tend to "spill over" from its preferred niche into others where it is less favored. Thus to some extent, frequency-dependent selective values should be expected on general ecological principles. By now, of course, it is well known that such frequency-dependent selection does exist. This has been shown for polygenic systems by Lewontin (1955) and for inversion systems by Dobzhansky, Pavlovsky and Levene (1954) and by Spiess (1957). The most important theoretical consideration arising in gene frequency-dependent selection is that the *stable equilibrium point may not coincide with an adaptive peak.* This is most easily demonstrated for a single locus, but applies in general. Suppose there are two alleles at a locus and the fitnesses of the three genotypes are:

AA	Aa	aa
1	1	$1.5 - q$

where q is the frequency of the allele *a*. In such a case the fitness of the genotype *aa* will decrease as its frequency increases and a stable equilibrium is reached at $q = .50$. However, the mean adaptive value at this equilibrium point is 1 while it is greater than 1 for lower frequencies of the allele *a*. For example, $\bar{W} = 1.016$ when $q = .40$. Thus, the saddle points for our *Moraba* populations may well represent points of stable equilibrium and indeed if these points prove to be stable, this is almost *prima facie* evidence of frequency-dependent fitness values.

The type of slope reversal shown in figure 12 might also be expected on meteorological grounds. It is, however, difficult to accept hypothesis IIc as the sole cause of the genetic equilibrium, since one would expect in this case that sooner or later a number of similar years would follow one another so that the population would evolve beyond the zone in which slope reversal occurs. Whenever this happens the population would thereafter continue to climb the ridge to A or B, in spite of any slope reversals in the region of the saddle. Again, the gradient is so flat in the region of the saddle that the populations might be stalled in this region for an indefinite period. Such a maintenance of an unstable equilibrium has been suggested by Li (1953) for the Rh alleles in human populations.

In summary, some combination of hypotheses IIb and IIc seems the most probable explanation of the observed apparent equilibrium of the inversion systems in *Moraba* with frequency-dependent selection being the more important of the two.

Summary

All the data currently available on the frequencies of inversions in chromosomes CD and EF of the grasshopper *Moraba scurra*, in certain populations in southern New South Wales, indicate an equilibrium condition in which the Blundell arrangement for chromosome CD and the Standard arrangement for chromosome EF predominate. Estimates of viability based on deviations of adult frequencies from binomial-square proportion show that there are epistatic interactions between the non-allelic arrangements in determining genotypic fitness.

From these estimates adaptive surfaces have been constructed which reveal that each population sampled is at a "saddle" or minimax point of the adaptive surface. The possible reasons for the apparent equilibrium condition of the populations at such an unstable point have been examined, and it is tentatively hypothesized that the equilibrium is maintained by a combination of gene frequency-dependent selection and yearly fluctuations in the physical factors of the environment.

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NOTES AND COMMENTS

A CRITIQUE OF "MORPHOLOGICAL INTEGRATION"

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Recently, Olson and Miller published, under the title "Morphological integration" (1958, Univ. Chicago Press), a method by which, they believe, new insight can be gained into the functional and evolutionary correlation of anatomical characters. The authors were concerned mainly with the exceedingly difficult problem of the degree of correlation between the many characters of a single functional unit or "character complex" in an animal and how this correlation changes during evolution, this being a problem that has puzzled and evaded evolutionists since Darwin. In an attempt to unravel the mysteries of character correlation, Olson and Miller developed an elaborate statistical method called the "ρF-model." The basic statistics of this method are correlation analysis. In their book, the authors describe the method of morphological integration and the ρF-model, present several studies to illustrate the type of results that may be gained through the use of this method and finally discuss the biological meaning of these results. Olson and Miller state frankly that they chose the book means of publication in order to direct the attention of more workers to the problems of morphological integration and because they felt that a smaller publication would not permit a complete presentation of their ideas. The importance of the problem and the complexity of the method certainly justify their decision. However, the appearance of "Morphological integration" leaves the student of evolution and of morphology with numerous problems. First, two published reviews of this book disagree on the usefulness of the ρF-model; Simpson (1958, Science, vol. 128, p. 138) feels that the huge amount of labor does not justify the results while Imbrie (1958, EVOLUTION, vol. 12, pp. 558–559) feels that, although the labor is great, the results could not be obtained by simpler means. Second, is the problem of whether the method is correct or applicable to the problems of the functional and evolutionary correlation of characters. The major difficulty of "Morphological integration" is that only a very few anatomists and evolutionists have the proper training to understand and evaluate the ρF-model—a problem well appreciated by the authors. Unfortunately no reviewer to date has discussed the statistics involved in the ρF-model; this will be the subject of my critique.

The basic hypothesis of morphological integration is that those structures which are functionally associated with each other will be statically correlated with one another. Thus, for example, if the length of the ulna is functionally associated with its width, then within the size range of the species, the longer ulnae would be the widest ones and the shorter ulnae would be the narrowest ones if that is the correlation. In accordance with this hypothesis, Olson and Miller assume that the greater the correlation, the closer is the functional association between two structures. That is, two structures having a correlation coefficient of .90 would have a closer functional association than two structures having a correlation coefficient of .30. The ρF-model was designed to ascertain the functional association between the structural features of an animal. Briefly, the steps in this method are as follows: (a) The structures to be analyzed are chosen and measured, the recommended sample size being 40 individuals; (b) The correlation coefficients of all possible pairs of structures (pairs of measures) are calculated; (c) The sample correlation coefficients "r" are transposed to the population correlation coefficients "ρ"; (d) Series of ρ-groups are formed, the "ρ's" having any arbitrary values —perhaps .85, .90, and .95 (A ρ-group is defined as a group of measures which are correlated to every other measure in the group with a correlation coefficient, "ρ", equal to or greater than the chosen ρ level); and (e) The basic pairs of measures (those pairs of measures which are more highly correlated to each other than to any other measures) are determined. The basic pairs are the "group formers" and are used to form the "basic pair ρ-groups." The basic pair ρ-groups are formed in such a way that there is no overlap between the different basic pair ρ-groups. Once these groups are formed, the use of statistics ends and the biological analysis begins. Thus, I shall concentrate my attention on the five steps just described.

These steps appear to be perfectly clear and reasonable when one reads the book, but upon closer examination, several subtle difficulties appear, especially in steps "c" and "e". These difficulties result from the fact that the structures being correlated vary only within the normal adult size range of the species, which might be very small as in the birds, and that the correlation coefficients being compared differ very slightly; the difference is often in the range of .10 to .05. Because of the fine differences used in the analysis, the ρF-model suffers from several

statistical weaknesses which can be grouped under two headings: (a) incorrect use of the null hypothesis; and (b) failure to establish confidence limits. These shall be discussed in turn.

Incorrect use of the null hypothesis. The first step in most statistical studies after obtaining the statistics is to test the probable validity of several null hypothesis associated with each statistic. For example, if a sample mean of 37 mm is obtained, a null hypothesis could be: If the sample was drawn from a population having a mean of 39 mm, is the probability of the observed deviation exceeding ±2 mm equal or less than 5%? If, when this null hypothesis is tested, a "t" value smaller than the appropriate "t" for the sample size and probability level is obtained, then this null hypothesis cannot be rejected. However, this does not mean that the sample was drawn from that population. In essence, Olson and Miller arrive at the latter conclusion—that the assumed population value is the correct one—when they outline (p. 12 *et seq.* and table 4, p. 49) how the sample correlation coefficient is transposed to the theoretical correlation coefficient for the population.

Olson and Miller point out (p. 46) that in actual studies, the sample correlation coefficient "r" is obtained rather than the desired population correlation coefficient "ρ" and give the following method for transposing the "r" values into the "ρ" values. For any "r" value, the corresponding "ρ" value is the maximum "ρ" value that is not significantly different from the "r" value at some chosen probability level. In fact, in their figure 4, they state, for some unknown reason, that for any "r" value, the corresponding "ρ" is the maximum "ρ"—or even greater "ρ"—that is not significantly different from this "r" value (e.g., if $r = .97$, then $\rho \geq .99$ and so on). Olson and Miller do not use this exact wording in outlining the method of transposing the "r" value to the "ρ" value, but this is clearly implied by their examples. Such a procedure is invalid and, as stated above, is a misuse of the null hypothesis. In fact, this procedure will consistently overestimate the population correlation coefficients.

A sample value, be it a sample mean, standard deviation or correlation coefficient, is only an estimate of the theoretical population value. The accuracy of this estimate depends upon the sample size. Thus "r" is a statistic and estimates "ρ" with a certain degree of accuracy which is measured by the confidence band. Hence, $(r - a) \leq \rho \leq (r + a)$, "a" depending on the sample size and the level of probability. For some reason, Olson and Miller ignore the negative side of the confidence band and state that $\rho = (r + a)$, or in their terms $r = (\rho - a)$. This statement is not necessarily wrong. Certainly it is possible that $\rho = (r + a)$; however, it is not probable. I could claim that for every "r" value calculated by Olson and Miller, the corresponding "ρ" is the minimum, not the maximum "ρ", or to make things more complicated, I could claim that the odd "r" values correspond to the maximum "ρ" value and the even "r" values correspond to the minimum "ρ" value. These claims would be fully as justified as Olson and Miller's, but they would prove disastrous for the ρF-model. However, "ρ" would most likely be neither the maximum nor the minimum possible value, but an intermediate value close to "r".

It is sometimes useful in statistics to choose the minimum or the maximum population value that does not differ significantly from the sample value. For example, if one wishes to prove that means of two samples are different, then for additional safety, one would choose the maximum theoretical population value for the smaller mean and the minimum theoretical population value for the larger mean. However, when a large number of sample values are being compared, such as in the ρF-model, the best value to use is the sample value. Olson and Miller do not give any reasons for using the maximum population correlation coefficients in their studies or why they cannot use the sample correlation coefficients. There is no statistical or biological reasons for choosing the maximum value. Indeed, if any value other than the sample correlation coefficient is to be used, it would be best to choose the minimum "ρ" value because the ρF-model depends upon proving a high degree of correlation between structures.

Failure to establish confidence limits. If statistics, such as sample means or sample correlation coefficients, are to be distinguished one from the other, it is first necessary to determine whether they are significantly different from one another at some chosen level of probability. If two statistics are not significantly different from one another, then the simplest null hypothesis is that the two samples were drawn from the same population. In other words, although there is a difference between these two statistics, one could not, with any assurance, distinguish between the populations from which the samples were drawn.

Olson and Miller (p. 47) define "basic pairs" as those pairs of measures that are more highly correlated with each other than to any other measure. The basic pairs are very important to the ρF-model because they form the separate "basic pair ρ-groups" which are the indicators of different functional complexes of characters. Different sets of basic pairs would form different groups of functionally correlated characters—thus the selection of the basic pairs is of prime importance to the results of a study. The only criterion for basic pairs is that they are more highly correlated to one another than to any other measure. Yet Olson and Miller do not determine whether the "r" values of the basic pairs are significantly different from the next higher "r" values of pairs involving one member of the basic pair. Many of the basic pairs cited by Olson and Miller differ from the next higher

pairs of measures involving one member of the basic pair by a difference in "r" of less than .05 and some by a difference in "r" of only .01, differences that are certainly non-significant. Rough calculations show that for samples in the range of 20 to 40 individuals, the "r's" must differ by at least .10 to .20 to be considered significantly different when they are in the range of .80 to .95. Usually the difference in "r" must be even higher, in the range of .30 to .50, before the "r's" between 0 and .80 are significantly different. Thus in studies of morphological integration, the "r" value of most basic pairs does not differ significantly from the "r" of many other pairs of measures involving one member of the basic pair. Consequently, one cannot, with any assurance, choose one pair of measures from a group of pairs whose "r" values are not significantly different and give it a special meaning —the basic pair. A second problem is that the basic pairs may not be the same if one repeats a study of morphological integration using a different sample. Therefore, I can only conclude that the definition of the basic pair does not have a sound statistical foundation. The consequences are obvious since the choice of basic pairs is an essential step in the ρF-model.

Conclusion. The two points in the statistical model discussed above are sufficient to invalidate the present ρF-model. First, instead of dealing with "high" levels of correlation, the levels are rather low; and second, the correlation coefficients can be distinguished from each other with a very low degree of precision, or conversely, the "r's" must differ by at least .10 to .20 before one can distinguish with any certainty between populations from which they were drawn. Since the entire argument of "Morphological integration" is built on the ρF-model, it can thus be no better than the model. Unless the authors can correct these points and present a statistically sound ρF-model, it can only be concluded that the method of "Morphological integration" is statically unsound and of little use in evolutionary studies.

Acknowledgments. I wish to thank the many people who have helped me during the course of this study. Special thanks must go to Dr. W. G. Cochran of the Statistics Department, Harvard University, who very kindly checked my statistics and to Dr. G. G. Simpson with whom I discussed the entire problem. Drs. Ernst Mayr, Carl Gans and Professor Bryan Patterson must be thanked for their aid in checking the statistics and reading the manuscript. These workers, of course, do not necessarily agree with all of the remarks contained in this paper and I am solely responsible for all errors in the statistics and the interpretation of "Morphological integration."

MORPHOLOGICAL INTEGRATION: A DISCUSSION

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Of the various reviews of Morphological Integration (Olson and Miller, 1958) that have appeared since its publication, a few have offered criticisms of certain statistical aspects of the study. Simpson (1958), for example, in his penetrating and thoughtful review comments as follows, "Their work depends heavily,—indeed fundamentally—on evaluation of differences between sample values of statistics, but the confidence levels and significances of these differences are established poorly, or not at all." Imbrie (1958) makes note of this comment as does Schaeffer (1959). F. N. David (1959), who comments as a statistician on the techniques and aims of the book, on the other hand, expresses views that lie very close to our own. The critique of Walter Bock, in this issue of EVOLUTION, makes it clear that there exist misunderstandings regarding our use of the sample correlation coefficient and the population parameter.

We regret that certain sections discussing these aspects have caused difficulty, and will attempt to clarify these parts.

The first paragraph of page 55, Morphological Integration, gives an explicit statement of the hypothesis tested, the confidence level α and the source of tables on which our work was based. We present this paragraph once again.

"We wish to test the hypothesis that $|\rho| \geq |\rho_0|$ versus the alternative $|\rho|$ is lower, where $|\rho_0|$ is some arbitrarily selected value of $|\rho|$. We reject $|\rho| \geq |\rho_0|$ when $|r| < c$ (lower limit of confidence interval for ρ_0). Let the sample size be n and the level of significance α. Then the probability $P_{|\rho 0|}\{-c \leq r \leq c\} = \alpha$ is given in table form in David (1938). For example, if $n = 25$, $\alpha = .05$ and $\rho_0 = 0.8$, then c from David's tables is 0.645. Suppose we wish to form the subset S such that all $|\rho_{ij}|$ are $> |0.8|$. We then accept all r_{ij} greater than or equal to 0.645. The form

of the sample matrix for the subset analogous to figure 6 is illustrated in figure 7." Attention is called to the statement $P_{|\rho 0|}\{-c \leq r \leq c\} = \alpha$. This states that the probability for an arbitrary level of correlation given $|\rho_0|$, that the sample correlation coefficient r lies between $-c$ and $+c$, is equal to α. For example, we consider a matrix array of sample correlation coefficients, and wish to select all those such that the probability that the population value $|\rho|$ is greater than or equal to 0.80 or conversely less than or equal to -0.80, is equal to $1 - (.05)$, or 0.95. Then for the given sample size of 25, we find that the critical level c is approximately 0.645. This means we accept sample values greater than 0.645 or less than -0.645, as drawn respectively from populations whose ρ is greater than or equal to .80 or less than or equal to $-.80$. This choice is dependent on three properties, namely, sample size, selected value of $|\rho_0|$ and α which, is explicitly the risk of rejecting the hypothesis for a given r, that $|\rho| \geq |\rho_0|$ when it is in fact true. We thus specifically take into account the sign of the correlation coefficient, the sample size, and the risk in the probability sense. It is our contention that the statistical treatment of r vs. ρ is correct and reasonable in view of the problem.

A number of misprints and errors have inadvertently appeared in the text. Several corrections are given here:

In the statement reprinted from p. 54 above the term (lower limit of confidence interval ρ), line 3, should be replaced by (critical level). The value 0.645, lines 6, 8, should be 0.601 within the accuracy of David's graphs. r_{ij}, line 7, should be $|r_{ij}|$. Table 4, p. 49, should read in the second column $\begin{matrix}|r| \geq \\ |\rho|\end{matrix}$ instead of $\begin{matrix}r \\ \rho \geq\end{matrix}$, wherever it appears in this column.

A comment on the basic pair method is also appropriate. For a fixed sample size the basic pair method for reduction of intersections between groups appears to be quite suitable. Its formal definition is given on p. 55 of the text of Morphological Integration. However, in cases where sample sizes vary within a single study (Olson, 1953) or from one study to the next, the results are not strictly comparable. For example, in several samples of a single species, the array of basic pairs may be somewhat different in each. The precise composition of the basic pairs is not important, but rather their group forming capacities. It is clear from studies such as that of the albino rat, described in the book, that the differences in precise composition of the basic pair array has little or no effect upon the groups formed in the several samples. In intent, the basic pair method is a numerical device to reduce intersections between groups. We fully agree that other methods may be more suitable, and have also used a changing level of $|\rho_0|$ for qualitative reduction of intersections in various chapters. However, at the time the work was carried out, no general solution to the problem of reduction of intersections of groups was known to us, and at present we still know of no such general solution.

A final word on the ρF model. The proposition contained in the model is essentially that there exist groups of highly associated morphological dimensions within most organisms—and that such groups have important biological and evolutionary meaning. This is formalized by stating that ρF groups are the result of contributions from the factors of function, development and a residual term (see p. 263 ff.).

Although we used the correlation coefficient ρ and its sample value r to assess degree of association to form these groups, the fundamental model does not depend on this or any particular statistical method. Other measurements of degree of association would, we feel, have given approximately the same results but were less practical for analysis of large collections of data. In fact, an initial investigation of the existence and possible biological interpretation of the groups made use of a mechanical system of "edge-marked" cards to assess degree of association, rather than the correlation coefficient (Miller, 1950; Miller and Weller, 1952).

That F groups exist, have biological meaning, and are of importance in evolutionary studies, we feel has been shown. The particular method of measuring degree of association, in order to form the groups, is secondary in importance, and undoubtedly subject to improvement.

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DETERMINATION OF DROSOPHILA KARYOTYPES FROM ADULT MALES

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The usual cytological technique for determination of the chromosome complements of Drosophila species is by examination of the large neuroblasts from larval brain tissue. Metaphase figures may be obtained by the standard squash preparations using either aceto-carmine or aceto-orcein. Mitotic chromosomes may also be analyzed from division figures among spermatogonial cells of the testes, prepared either by sectioning and staining the gonad or by squash or smear techniques (Darlington and LaCour, 1942). Although actively dividing spermatogonia are most abundant during pupal development, it is possible to obtain mitotic figures in mature adult males from the apical region of the testis where cysts of spermatogonia continue to divide.

Testes were dissected from mature adult males in Drosophila saline solution; the apical region containing spermatogonia was removed and transferred directly to aceto-orcein for twenty minutes. The gonad was squashed in 50% acetic acid, and the cover glass sealed in place with a 1:1 mixture of petroleum jelly and paraffin. Such preparations could be scanned rapidly for the presence of dividing spermatogonia. The apex of the testis is easily located and with a little practice the right amount of pressure needed to rupture the testis and give clear metaphase configurations may be determined.

The males of eight species, all stocks from the Genetics Foundation at the University of Texas, were examined for larval brain configurations and spermatogonial metaphases from adults of different ages: *D. melanogaster,* 7 days; *D. virilis,* 2 days and 7 days; *D. americana,* 2 days; *D. affinis,* 3 days; *D. repleta,* 2 days and 7 days; *D. ananassae,* 2 days; *D. camargoi,* 7 days; *D. mediostriata,* 7 days. Satisfactory configurations were obtained for all species examined from both larval brain preparations and from the adult testes at the ages indicated. Camera lucida drawings were made of metaphase configurations from larval neuroblasts and from the adult spermatogonial cells for comparison of size and shape of the chromosomes. Drawings from four of the species are shown in figure 1.

In species which mature slowly, the metaphase configurations are found with little difficulty. In *D. melanogaster* and other species which mature rapidly, division figures are more sparse. This technique may be of particular value in studying the chromosomes of those species collected from wild populations which

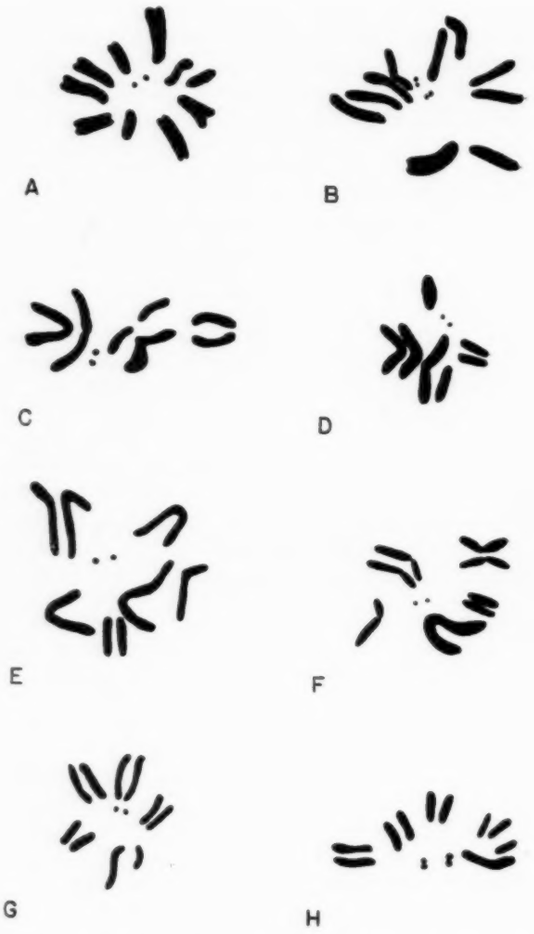

FIG. 1. Metaphase configurations of several species of Drosophila (Original magnification, 900×; camera lucida drawings). A—*D. virilis* male, larval neuroblast; B—*D. virilis* male, spermatogonial cell from 7-day adult; C—*D. americana* male, larval neuroblast; D—*D. americana* male, spermatogonial cell from 2-day adult; E—*D. affinis* male, larval neuroblast; F—*D. affinis* male, spermatogonial cell from 3-day adult; G—*D. repleta* male, larval neuroblast; H—*D. repleta* male, spermatogonial cell from 7-day adult.

do not thrive under laboratory conditions. If adult males are available, and larvae do not develop in the laboratory, it may be possible to determine the karyotype of a species by using this method.

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SUPERNUMERARY CHROMOSOMES IN *HAPLOPAPPUS GRACILIS*

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Since reporting the chromosome number of *Haplopappus gracilis* as $n = 2$ (Jackson, 1957), I have found other chromosome numbers in this species. In a collection of plants grown from seed obtained at Bandelier National Monument in New Mexico, chromosome numbers of $n = 2$, $n = 2 + 1$, $n = 3$, and $n = 3 + 1$ were counted. Plants having more than the normal two pairs of chromosomes were found to contain supernumerary chromosomes in the usual sense in which this term is used.

In all of the New Mexican plants studied, the supernumerary chromosomes were morphologically identical. Each chromosome possessed a near-median centromere and was approximately two microns in length at mitotic metaphase. This is about one-half the length of chromosome B and one-third the length of chromosome A of *H. gracilis*. Studies of diakinesis failed to show any pairing of the supernumeraries with the A and B chromosomes.

In the few plants grown thus far, there have been certain morphological characteristics associated with plants carrying the supernumerary chromosomes. In some plants the type of pubescence was slightly altered and the branches were thicker, especially near the tips, giving a "polyploid" appearance. In one plant, there was a greater distance between the spines on the leaves than is found in normal individuals. In typical plants of *H. gracilis* the achenes are brown or reddish, but in plants carrying the supernumerary chromosomes the achenes were a dark purple. It thus appears that the supernumerary chromosomes may exert a decided genetic effect, depending, perhaps, upon the genotypes with which they are associated. The characteristics associated with the supernumerary chromosomes were noticed first, and a later cytological study revealed the presence of the extra chromosomes.

In addition to the supernumeraries found in plants from New Mexico, others were found recently in a population of plants from southwestern Arizona. Some plants from this population contained up to four supernumerary chromosomes that were much shorter than those in the New Mexican material. Seeds from this population are being grown in order to further analyze the material cytologically and genetically.

Various explanations have been offered for the origin of supernumerary chromosomes in plants (Lewis, 1951; Swanson, 1943). In *H. gracilis*, I believe that two methods of origin may be possible. One would be the introduction of extra chromosomes into populations of *H. gracilis* as a result of hybridization and backcrossing with a species having a larger chromosome number. The second possibility might be that the extra chromosomes represent the centromeres and adjacent chromatin resulting from the aneuploid reduction process that may have given rise to the species. The mechanisms of this process have been outlined by Darlington (1937) and Stebbins (1950). It is generally assumed, however, that the centromeres and adjacent chromatin will be lost if they are genetically inert. Nevertheless, it may be possible that the centromere and adjacent chromatin are retained in some populations of the species even though they are inert and unnecessary for normal development.

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NATURAL SELECTION IN MIDDLE ISLAND WATER SNAKES (*NATRIX SIPEDON* L.)

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In an earlier paper (Camin and Ehrlich, 1958) postnatal selection for pattern type was demonstrated in water snakes living on the islands at the western end of Lake Erie. Comparison of pattern type frequencies in litters with those in the adult population showed a statistically highly significant difference on the islands grouped as the "Bass Complex." However, although the data were suggestive, no significant difference was found between litter and adult populations on Middle Island. This appeared to be due to insufficient sampling (3 litters only).

In early June of 1958 the authors, accompanied by Dr. R. W. Hull of Northwestern University and Dr. W. J. Beecher of the Chicago Academy of Sciences, again visited Middle Island. In two days of collecting 84 mature and 20 juvenile snakes were captured. Nine litters, ranging in size from 2 to 31 individuals, were obtained from the captive snakes. In seven of these litters the female parent was of a class closer to the unbanded extreme of the distribution than was the median class of the offspring. In the other two litters the female was from the same class as the litter median.

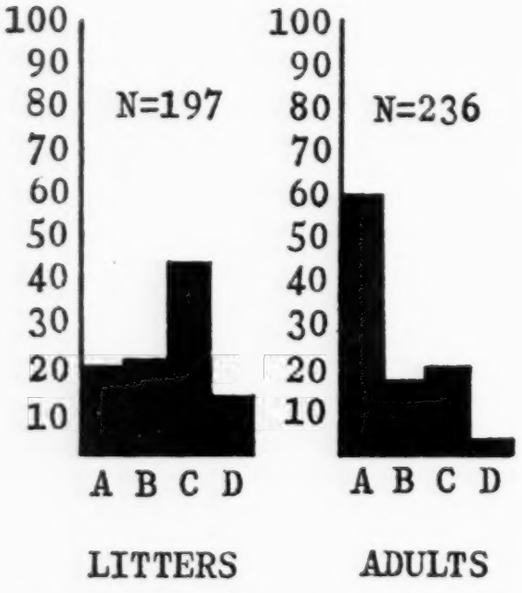

FIG. 1. Comparison of litter and adult populations (ordinates—per cent of total; abscissas —pattern types).

Figure 1 shows the distribution of pattern types (coding as in Camin and Ehrlich) of Middle Island adults (data from 1949, 1957 and 1958) and those of the litters (data from 1957 and 1958). The adult and litter frequencies were compared statistically using the ultra-conservative test outlined by Calhoun (in Camin and Ehrlich, 1958), and no significant difference was found. However, the somewhat less conservative methods of Hansen, Hurwitz and Madow (1953) showed a significant difference in both one-tailed ($P = .008$) and two-tailed ($P = .016$) tests.

The additional litters brought our total from the Lake Erie Islands to 23, permitting us to test statistically our previous assumption that litter size and pattern frequency are independent. Using a 2×2 table to test for independence we obtained an adjusted χ^2 value of .127 ($.50 > P > .40$) which, indeed, supports our assumption.

It is of some interest to note that if we divide the pattern spectrum into only two classes, the young snakes in the heavily banded half of the litter population have a chance of survival which is only about 23% of that enjoyed by those in the relatively unbanded half.

We regret to report that Middle Island, which was uninhabited for many years, is now once again occupied in the summer months. A campaign of extermination is being waged against the snakes, and for this reason we felt it would be foolish to initiate our planned program of marking and releasing snakes for long-term study.

We wish to thank Dr. R. N. Bradt and Dr. D. R. Truax of the Department of Mathematics, University of Kansas, for advice on the analysis of our data.

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CAMIN, JOSEPH H., AND PAUL R. EHRLICH. 1958. Natural selection in water snakes (*Natrix sipedon* L.) on islands in Lake Erie. EVOLUTION, 12: 504–511.

HANSEN, M. H., W. N. HURWITZ, AND W. G. MADOW. 1953. Sample Survey Methods and Theory. Vol. 1. J. Wiley. (chapter 4).

POSSIBLE EVIDENCE OF RAPID EVOLUTION IN HAWAIIAN MOTHS[1]

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The speed of evolution of species is a subject of much speculation and debate, and concrete evidence of rates of change is meager. Buried in the depths of the eighth volume of my series *Insects of Hawaii* (Lepidoptera: Pyraloidea, ix + 456 pp., 347 figs., University of Hawaii Press, Honolulu, 1958) is a discussion of possible "rapid evolution" that may interest some readers of *Evolution* but which probably will escape the notice of those who do not have special reason to refer to my text. Some of my friends, therefore, have urged me to publish this note to call attention to the problem as I have found it.

Polynesian man settled the Hawaiian Islands from the Tahiti area about 1,000 years ago. He brought with him the banana, amongst several other food plants. There were no bananas or banana relatives in Hawaii when man first arrived. Moreover, there are no bananas growing natively on any of the islands of the central Pacific Basin. All bananas on Polynesian islands have been established there by man. The nearest bananas growing endemically are found several thousand miles southwest of Hawaii in the Indo-Australian region.

About 1,000 years ago, then, the banana was newly established in Hawaii. Between then and now there have been developed several species of Hawaiian pyraustid moths of the genus *Hedylepta* that evidently are obligatory feeders on the foreign banana. After extensive study over many years, mostly by O. H. Swezey, it has been ascertained that five known species of *Hedylepta,* and others as yet undescribed, are confined to banana and are not known to attack any other plant. These species form a closely linked group of endemic Hawaiian moths which have evolved from endemic species. They were not introduced by the Polynesians. There is no question of their endemicity or the fact that they have evolved in Hawaii. *Hedylepta* is a large genus that ranges natively from Africa to Polynesia. The larvae are mostly leaf-rollers. Twenty-three species have been described from Hawaii, and larvae of some undescribed species have been discovered. All of these species are Hawaiian endemics; no species of the genus occurs in Hawaii and elsewhere.

[1] Written during the tenure of a grant from the National Science Foundation to the University of Hawaii for the project "Insects of Hawaii."

Now we are brought to face our problem and the reason for this paper. If, as we have concluded, these banana-eating species of *Hedyleptc* are confined to banana, then they evidently must have evolved on banana during its short history in Hawaii, and we have here a probable case of a species complex of five or more species evolving upon a new hostplant within about 1,000 years. These species are differentiated by characters of the adult moths as well as the immature stages, and the pre-adult differences are in some instances of greater intensity than are those of the adults.

An outline of the hostplant groupings of the 23 described Hawaiian species is as follows: six species feed on grasses; five on banana; four on sedges; three on lilies; two on palms; one on *Joinvillea* (Flagellariaceae); one on legumes; one unknown, but grass is the presumed host. It will be noted that all but one species are attached to monocotyledons.

The five species of the banana complex are closely allied to the two species that feed on palms, and the entire group of seven species makes up a closely interlinked species cluster. One of the palm-eaters (the most common and widespread *blackburni*) occasionally attacks banana. The only native Hawaiian palms belong to *Pritchardia.* We know from fossil and other evidence that *Pritchardia* palms originally were widespread in Hawaii from near the seashore to the highlands, but, with a few, isolated, little-known exceptions, all of the lowland *Pritchardia* have been exterminated by fire, cultivation and other activities of man. (It is of interest to note that fossil *Pritchardia* have been found in excavations below present sea level, thus clearly indicating submergence of the land in Hawaii).

When man first arrived in Hawaii, *Pritchardia* was common and widespread, and presumably attached to the palm was a species of *Hedylepta.* I believe that it was this *Pritchardia*-eating *Hedylepta* that gave rise to the group of species that we now find on palms and bananas. We may consider that after man introduced the coconut palm and the banana, the ranges of these two plants expanded as the endemic *Pritchardia* retreated, and with the retreat of the palms to extinction over large areas, *Hedylepta* took to feeding upon the coconut and the banana. One of these, *Hedylepta blackburni* (Butler), or its antecedent, became attached principally to coconut palm in the

lowlands, and a number of closely related species developed on banana in various forested and higher parts of the islands. All of the species of the complex under discussion are allied closely to *blackburni,* and that species may represent the ancestral stock. This group has a distinctive type of wing marking that separates all of them from all other species of the genus in Hawaii. *Hedylepta blackburni,* the coconut-eater, is widespread in the lowlands of all of the main islands, but its wide distribution probably has been assisted greatly by man who regularly carried coconut palm fronds between the islands. The five described banana-eaters are much more limited in their distributions and are found only in the mountain forests. One of these is confined to the adjacent islands of Kauai and Oahu; one is known only from the closely adjacent islands of Maui and Molokai, and the remaining three species, plus the species confined to *Pritchardia,* are found only on the island of Hawaii. (I am not aware, however, that any two of these species occupies the same area. It would be of interest to investigate the possibility that the isolated populations of the two species recorded as each inhabiting two islands may be racially distinct.)

In summary, then, we may say that about 1,000 years ago endemic *Pritchardia* palms were widespread in the Hawaiian lowlands, and there were no bananas or coconut palms in Hawaii until they were introduced by Polynesian man. It appears that within about 1,000 years at least five new, closely allied species of *Hedylepta* moths have been developed on the banana from an endemic ancestral stock that probably fed upon lowland *Pritchardia* palms. If this statement is challenged, then we must produce an explanation for the fact that these species are attached entirely to the introduced banana. Why do they have no other hostplants? (I should point out that the moths do not feed on bananas because there are no palms available, because they confine themselves to banana even when the banana is growing in the vicinity of palms.) These banana-eaters appear confined to the old, wild Polynesian varieties of banana, and I have no records of their attacking the more recently introduced, cultivated varieties of market bananas. On the other hand, the common, widespread *Hedylepta blackburni,* that may be the stem form of this group, although mostly attached to the coconut but occasionally feeds upon *Pritchardia* and other palms and wild bananas, has on a few occasions been found eating modern varieties of cultivated bananas. Further details, including illustrations, are included in *Insects of Hawaii,* as referred to above.

Such problems as these in the great "insular laboratory" of Hawaii deserve further study. Unhappily, however, the time when such research could best have been made has passed. The pressure of foreign parasites on these moths has been extreme, and where once *Hedylepta* were common in the mountains, few or none can be found today. I know of only three endemic parasites of *Hedylepta* in Hawaii, and they exerted only light pressure. Now, however, at least eight foreign parasitic wasps and three foreign parasitic flies, mostly introduced purposely by agriculturists, have placed extraordinary and devasting pressure on the eggs, larvae and pupae of *Hedylepta.* Parasitism commonly exceeds 90%, and all eggs, larvae and pupae in some *Hedylepta* colonies evidently may be destroyed by these foreign parasites, and we know almost nothing about the introduced insect diseases that attack the immature stages. Most species of Hawaiian *Hedylepta* are now scarce or rare insects, and a number of species have not been seen for many years and may now be extinct together with untold numbers of other Hawaiian animals and plants.

Hawaii is a marvelous assemblage of gigantic volcanic mountains that, because of the activities of man, for long has been filling rapidly with the ghosts of its extraordinary and wonderfully evolved biota. Much of the living biota and facts concerning it continue unrecorded. Although it will be difficult, many problems such as that of *Hedylepta* may still be pursued with profit, but the few persons who are aware of the situation have been mostly crying in an unhearing wilderness and have been unable to carry on such research because of the impossibility of obtaining sponsorship in Hawaii where there has been a shift of emphasis in some quarters to "more practical work."

ORIGINS OF ANGIOSPERMOUS PLANTS

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In the June, 1959 issue of Evolution a review was published of the booklet, "Origins of Angiospermous Plants," by A. L. Takhtajian, which was translated from the Russian under my editorship. The reviewer, Dr. Verne Grant, recommended the work as a general summary

of its field, but criticized it for an incomplete citation of sources. The documentation was stated to be "on the level of a teaching text rather than a scholarly work." In response to this criticism Dr. Takhtajian has pointed out in a letter that the booklet was really meant to be a teaching text, as was evident from the format of the original Russian edition.

Dr. Takhtajian believes that Dr. Grant's review will create the impression that he does not give sufficient credit to the work of botanists in western countries. Consultation of the research papers by Dr. Takhtajian, several of which are cited in the literature list of "Origins of Angiospermous Plants," will quickly correct this mistaken impression. The more recently translated work of Dr. Takhtajian, "Essays on the Evolutionary Morphology of Plants," has abundant references to western literature (see review by Grant immediately following). A non-Russian bibliography of 186 items was omitted from the translated text of this work in order to save space. For the same reason we did not reprint Takhtajian's list of western literature in the translation of "Origins of Angiospermous Plants."

The Russian edition of "Origins of Angiospermous Plants" has the aspect of a textbook summarizing the most recent information for the benefit of students. My reasons for having it translated in spite of its semi-popular character were, first, that I know of no publication in the English language in which all of the recent information regarding the origin of Angiosperms has been brought together and integrated as it is in Takhtajian's volume; and, second, that its publication would make English speaking botanists aware of the fact that botanists in the USSR have developed ideas about this intriguing and basic problem of plant science which closely resemble those held in western countries, and would serve to open up avenues of communication between them and those botanists unable to read the Russian literature.

The English edition was published by the American Institute of Biological Sciences in its series of Russian monographs; the semi-popular character of the work was unavoidably lost in translation. Most botany students and many instructors in the English-speaking world might consequently assume that "Origins of Angiospermous Plants" is an original work, even a statement of a new general theory, unless the facts are pointed out to them. Grant's review, which dealt specifically with the English edition of Takhtajian's booklet, was an attempt to view this work in its proper perspective. Since the standards of documentation vary widely in semi-popular writing, different readers may be expected to have different opinions about the adequacy of the citations of sources in the "Origins of Angiospermous Plants," even considered as a textbook.

In any case Dr. Grant informs me that he shares with Dr. Takhtajian the hope that "Origins of Angiospermous Plants" will not be regarded, on the basis of his review, as being on the same level with Dr. Takhtajian's scientific writings of a research nature, and that he shares with me the hope that the English translations of Takhtajian's works will help bring together the ideas and research of Russian and Western botanists working in the field of plant evolution.

EVOLUTIONARY MORPHOLOGY OF PLANTS[1]

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Professor Takhtajan has undertaken in the present work to examine certain fundamental problems of evolutionary plant morphology in the light of a neo-Darwinian philosophy. He carries out his task in 129 pages of text written with a depth of understanding of both plant morphology and evolutionary processes.

Takhtajan begins his theoretical discussion by distinguishing between progressive evolution and trends of specialization. "While general progress of organization has, so to speak, a universal nature and consists of alterations of a very general character, which are retained in their most basic traits for a long time and even in the course of the subsequent progressive evolution, the particular adaptations are, on the contrary, unstable in the subsequent evolution." (p. 36.) "In the course of evolution of both the plant world and the animal world a period of general progress is invariably followed by a period in which formation of particular adaptations prevails. . . . There takes place an adaptive irradiation. . . ." (p. 37.).

He next points out that evolutionary trends may proceed at different rates in different parts

[1] Takhtajan, A. L. Essays on the Evolutionary Morphology of Plants. (Transl., Amer. Inst. Biol. Sci., Washington, 1959; original Russian edition, Leningrad University, 1954).

of the plant body. Thus the flower of *Delphinium* possesses some primitive features (free carpels) combined with some advanced features (zygomorphic corollas). These "evolutionary age differences of characteristics" are more pronounced in the plant kingdom than in higher animals because of the lower degree of coordination between different parts of the body in plants, which develop by an open system of growth. Where separate characteristics are functionally linked in plants, as in the different features of wood anatomy, these characteristics tend to evolve together and hence at similar rates. Thus is offered an explanation for a problem which has vexed students of plant phylogeny, namely that a correlation between different primitive (or advanced) characters may be present in one case but conspicuously absent in another.

In Chapter 5, Takhtajan explores critically the idea that phylogenetic changes come about through successive modifications of ontogeny. Whereas changes occurring in the final stages of ontogeny will usually lead to minor alterations in the adult form, deviations in early ontogeny may initiate far-reaching evolutionary changes. Of widespread significance is the fixation of some juvenile stage as a new adult stage (neoteny). Many examples are cited. Thus Duckweeds (*Lemna*) are in the adult stage like seedlings of the aroid, *Pistia;* by neoteny the Lemnaceae evolved from Araceae (p. 62). The origin of herbaceous from woody angiosperms is neotenous, the stems of herbs being comparable to the first annual rings of their woody ancestors. Many woody plants have been known to flower as seedlings, as happened once with a mahogany 25 cm. high (p. 60). Consequently the transformation from tree to herb is not necessarily gradual, going through the stages of shrub and semi-shrub, but can be relatively abrupt.

Specialization, involving a restriction of the possibilities for future evolutionary change, may and often does lead into a blind alley. It would perhaps always do so "if organisms were to evolve only through alteration of the final stages of their development. But owing to the fact that evolutionary alterations can occur at any stage of ontogeny, the organism has at its disposal the means of extricating itself from this 'impasse of specialization.'" (p. 64.) Neoteny offers a way out of the blind alley of specialization (as emphasized independently by A. C. Hardy in *Evolution as a Process* (1954) and previously by Garstang).

The *Essays on the Evolutionary Morphology of Plants* reflects original thinking on such subjects as the value of teratological materials, the biogenetic law, and so forth. It mainly achieves originality, however, through the establishment of new connections between old facts familiar enough in some quarters and concepts current in other quarters. Takhtajan has broken down the isolation between much strictly botanical thinking and much evolutionary thinking.

He states: "At the present time, the idea of evolution through alteration of the course of individual development is penetrating ever more deeply into evolutionary animal morphology. As for botany, for a long time nobody has systematically applied this idea, although individual ideas in the spirit of phylembryogenesis have not infrequently been expressed by investigators in various fields of morphology. This can be attributed to the general backwardness of the theory of plant morphology as compared to evolutionary animal morphology. . . . A great many general ideas voiced in zoology have subsequently also proved applicable to plants and have thus acquired an all-biological significance." (p. 50.) This comment, applied here to one particular topic, is applicable to every other morphological problem discussed in this book.

The *Essays on the Evolutionary Morphology of Plants* is in an entirely different category from the *Origins of Angiospermous Plants*, which I reviewed in EVOLUTION, June 1959. With regard to the last-mentioned work, I noted that its documentation was on the level of a teaching text rather than on that of a scholarly work. As Professor Takhtajan later pointed out in a letter, the *Origins of Angiospermous Plants* was really meant to be a teaching text, although this would not be evident to readers of the English edition, which was published in a series of monographs. In any case the *Essays on Evolutionary Morphology* is in the scholarly tradition. It synthesizes the findings and views of the classical western morphologists, of many relatively unknown Russian workers, and of contemporary students in all lands. (The non-Russian biblography was unfortunately omitted in the translation in order to save space.) The resulting synthesis, brought across the language barrier by the translator, editor and publisher of the English edition, will undoubtely stimulate research and thinking in the English-speaking world on the morphological aspects of plant evolution.

MOLECULAR BIOLOGY [1]

G. COHEN-BAZIRE

University of California, Berkeley

In the past few years, the central problem of biology at the cellular level—namely, the structural, synthetic and functional interrelationships of nucleic acids and proteins—has become amenable for the first time to fruitful experimental study. The necessary methods and concepts were developed by scientists from a number of different fields, in connection with investigations which, at least initially, often did not seem to be interconnected. The linking up of these diverse lines of work has led to the emergence of a new field, which is becoming known as "molecular biology." Dr. Anfinsen has provided the first general account of the major accomplishments to date in molecular biology. Since this is certainly the most active, and perhaps the most important field of contemporary biological research, he deserves our gratitude.

The subject proper of the book is preceded by two chapters which provide brief and elementary accounts of evolutionary concepts and of the principles of classical genetics. They could well have been omitted. For those who are biologists, the information which they provide is superfluous; for those who are not, it could have been found, much better presented, in many elementary texts. The main body of the book falls into three principal sections. Two chapters describe the properties of genetic material, considered from both the molecular and biological aspects: the synthesis and structure of desoxyribonucleic acid, and the fine structure of the gene, respectively. Then follow three chapters on proteins covering general protein structure, the relation of structure to biochemical function, and the range of structural variation in specific proteins isolated from different organisms. The third section consists of three chapters dealing with various aspects of the problem of protein synthesis: the genetic determination of protein structure; the accuracy of the synthetic process; and its biochemical mechanism. These eight chapters provide a detailed, comprehensive and up-to-date account of the important experimental findings of the past decade. The final chapter of the book is a terminal essay of more speculative character, in which Dr. Anfinsen stakes the claim for molecular biology as a new and powerful tool in the study of evolution. General biologists will probably feel that the evolutionary problem has been made to appear a good deal more simple than it really is; but if one is willing to overlook this limitation, the essay makes good reading. The methods of molecular biology are evidently well suited for detailed analysis of microevolutionary events; but they are not apt for the study of macroevolution.

[1] Anfinsen, Christian B. 1959. The Molecular Basis of Evolution. xiii + 228 pp., 9 figs. $7.00. John Wiley, New York.

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Wallace, Bruce and Th. Dobzhansky. 1959. Radiation, genes, and man. xii + 205 pp. 19 figs. (Price not stated.) Henry Holt. An evaluation of radiation as a source of genetic damage to man, addressed largely to the general reader.

Haber, Francis C. 1959. The Age of the World. xi + 303 pp. $5.00. Johns Hopkins Press. An account of the historical and philosophical bases of the transformation of the views on the age of the world from those based on the time scale of the Bible to those of the 1860's.

Oncley, J. L. (Editor-in-Chief). 1959. Biophysical Science—A Study Program. viii + 568 + 14 + 27 pp. Illus. $6.50. John Wiley. A series of 61 articles by many authors including a number on the genetic aspects of nucleic acids.

REPORT ON THE FOURTEENTH ANNUAL MEETING OF THE SOCIETY FOR THE STUDY OF EVOLUTION

HERBERT H. ROSS

MINUTES OF THE BUSINESS MEETING

The meeting was called to order at the Hotel Shoreland, Chicago, Illinois, at 4:15 P.M., November 27, 1959, with Vice President Harlan Lewis in the chair. Approximately 70 members were present.

1. The minutes of the last business meeting, held at The University of Michigan, September 2, 1958, stood approved as published.

2. The Secretary reported on the activities of the Society during the year, listing various committee appointments. He announced that the Commissioners of Internal Revenue for Great Britain have approved the Society for the Study of Evolution for the purpose of allowing income tax relief on Society dues, as of October 26, 1959. British members have been notified.

3. The Secretary announced the results of the election for officers and councillors for the Society for 1960.

4. The chairman summarized the report of the Editor, pointing out that EVOLUTION has continued as a flourishing international journal but that its continued increase in size has caused a budget deficit over the last several years.

5. The Treasurer summarized his audited report for the year ending December 31, 1958, and also the interim report for 1959. He pointed out that our rising expenditures are considerably in excess of dues and subscriptions and are somewhat counterbalanced only by a rise in the sale of back issues. This sale, however, does not keep pace with the rising expenditures.

6. The Secretary summarized the reports received and the action taken at the Council Meeting:

(a) The following Associate Editors were appointed for a 3-year term: David G. Catcheside, Birmingham, England; Verne Grant, Claremont; Richard C. Lewontin, Rochester; Sajiro Makino, Sapporo, Japan; J. N. Spuhler, Ann Arbor; Mogens Westergaard, Copenhagen, Denmark.

(b) A special committee appointed last year to investigate ways and means of reducing the work load of the Treasurer's Office reported that the simplest method of accomplishing this was to use the business management services of the AIBS. This would cost the Society about $1.75 per account and aggregate about $3000 per year. The committee also reported that EVOLUTION could be published in Europe at a possible saving of approximately $3000 per year.

(c) In discussion the Council felt that it was imperative to reduce the work load of both the Treasurer and Editor and voted to use the AIBS services for both business management and redactory service, the latter at a probable cost of $1200 per year.

(d) In further discussion of finances the Editor pointed out that the already great problems in keeping EVOLUTION going and on time would be vastly increased with a foreign printer and that problems concerning storage and mailing of back issues, possible loss of manuscript materials and other items were still unresolved. On his recommendation the Council voted to continue publication of EVOLUTION with Lancaster Press for another year.

(e) The Council approved a budget of $12,500 for EVOLUTION.

(f) To meet the increased budget, the Council voted by 3 to 1, with 1 abstaining, to recommend that, effective for 1961, the annual dues be raised $2.00 per year. It voted unanimously that the subscription rate be set $2.00 higher than the dues and that a revised scale of increased prices for back issues be established.

(g) The Council also instructed the Secretary and Treasurer to cooperate in submitting to the Council a list of policy items designed to improve the Society's financial position.

(h) The Council authorized an extensive membership drive committee under the chairmanship of the Secretary to increase the field representation of the Society.

(i) The 1961 annual meeting of the Society will be held at Purdue University, Lafayette, Indiana, August 1961, in conjunction with those of the AIBS.

7. On behalf of the Council, the Secretary moved (a) to amend By-laws, Article 1, Dues, first sentence, by changing $6.00 to $8.00, and (b) that this be effective as of January 1, 1961. Motion seconded. Discussion from the floor stressed the importance of keeping the dues at a minimum in order to encourage membership in countries at a dollar disadvantage and brought out the decided sentiment that the meeting felt that the Council should explore further possibilities of reducing the operating costs rather than raising the dues. Suggestions from the floor included soliciting members for donations, a drive to sell sustaining memberships, dividing offices to make use of volunteer

workers, and many other constructive ideas. Motion defeated.

8. It was moved and seconded that this meeting wished the Constitution changed to provide life memberships at a cost of about twenty times annual dues and that an endowment fund be established to provide a source of growing revenue for the Society. Motion carried and referred to the Secretary for appropriate action.

9. It was moved and seconded that the By-laws be amended to raise the annual dues $1.00, to be effective in 1961. Motion carried.

10. On behalf of the Council the Secretary moved (a) to amend By-laws, Article 2, Publications, second sentence, by changing $7.00 to $9.00, and (b) that this be effective as of January 1, 1961. Motion seconded. It was moved to amend the motion by changing $9.00 to $10.00. Motion carried as amended.

11. A motion of appreciation for the facilities and assistance arranged by the Local Arrangements Committee, the University of Chicago Darwin Centennial Committee, and the Shoreland Hotel was approved by acclamation.

The meeting adjourned at 5:30 P.M.

HERBERT H. ROSS, *Secretary*

PROGRAM

The program of papers consisted of two sessions each on November 25 and 27. Drs. W. Frank Blair, Harlan Lewis, Herbert P. Riley, and Charles H. Seevers served as chairmen.

Invitation Papers

CARSON, HAMPTON L., Washington University, St. Louis, Missouri. Homoselection Versus Heteroselection and the Origin of Species.

MOORE, THOMAS E. and RICHARD D. ALEXANDER, University of Michigan, Ann Arbor. The Evolutionary Relationships of 17-Year and 13-Year Cicadas.

Contributed Papers

ALEXANDER, RICHARD D. and ROBERT S. BIGELOW, University of Michigan, Ann Arbor, and MacDonald College, Quebec, Canada. Allochronic Speciation in Field Crickets.

BADER, ROBERT S. and JOHN S. HALL, University of Illinois, Urbana, Osteometric Variation and Function in Bats.

BARRETT, PAUL H., Michigan State University, East Lansing. A Bibliography of Literature Cited in Darwin's Notebook on "Transmutation of Species."

BLAIR, W. FRANK, University of Texas, Austin. The Speciational Process as Indicated by Allopatric Populations of Anuran Amphibians.

BROOKS, HAROLD K., University of Florida, Gainesville. Precambrian Life and the Origin of the Cambrian Fauna.

BROWER, JANE VAN ZANDT, Amherst College, Massachusetts. Experimental Studies of Mimicry.

BROWER, LINCOLN P., Amherst College, Massachusetts. Experimental Investigation of the Origin of Migration in the Monarch Butterfly, *Danaus plexippus* L.

BURNS, JOHN M., University of California, Berkeley. Evolution in Skipper Butterflies of the Genus *Erynnis*.

CARPENTER, JOHN M., University of Kentucky, Lexington. The Role of Yeasts in Seasonal Fluctuations of *Drosophila* Species.

CRENSHAW, JOHN W., University of California, Berkeley. Paper Electrophoretic Analysis of Serum Proteins of Interspecies Turtle Hybrids.

DUELLMAN, WILLIAM E., University of Kansas, Lawrence. Comments on the Evolution of the Middle American Herpetofauna.

EDGREN, RICHARD A., Northwestern University, Evanston, Illinois. Geographic Variability in the Hog-Nosed Snake, *Heterodon platyrhinos* Latreille.

EHRMAN, LEE, Columbia University, New York, New York. Hybrid Sterility in *Drosophila paulistorum*.

EPLING, CARL, University of California, Los Angeles. Four Components of Adaptability that Stem from Inversions.

FEUER, ROBERT C., University of Michigan, Ann Arbor. Stabilizing Selective Mortality with Special Reference to Snakes.

GANS, CARL, University of Buffalo, New York. Pattern and Behavioral Mimicry in an African Snake.

HALL, E. RAYMOND, University of Kansas, Lawrence. Geographic Distribution of North American Mammals.

HAMILTON, TERRELL H., Harvard Biological Laboratories, Cambridge, Massachusetts. Adaptive Trends of Variation in Wing Length within Bird Species.

HAPP, GEORGE B., Principia College, Elsah, Illinois. Evolution Supported by Ecology.

HARRELL, BYRON E., State University of South Dakota, Vermillion. The Avifauna of the Sierra de Tuxtla, Mexico, and its Biogeographic Significance.

JOLLIE, MALCOLM, University of Pittsburgh, Pennsylvania. Relationships within the Holostei, a Comparison of *Amia* and *Lepisosteus*.

KENNERLY, THOMAS E., JR., Baylor University, Waco, Texas. The Role of Microclimate in Speciation.

LEVINS, RICHARD, Columbia University, New York, New York. Fitness in a Heterogeneous Environment.

MacDonald, Daniel J., Dickinson College, Carlisle, Pennsylvania. Adaptive Values in Experimental Populations of *Tribolium confusum*.

Milstead, William W., University of Kansas City, Missouri. Interrelationships between Two Species of Canyon Lizards.

Moore, Joseph Curtis, American Museum of Natural History, New York, New York. Zoogeographic Inferences from Observed Morphological Relationships in Living Squirrels of the Sciurinae.

Riley, Herbert P., University of Kentucky, Lexington. Paper Chromatography as a Possible Tool for Studying Evolution.

Robertson, George M., Grinnell College, Iowa. Phylogenetic Implications of Recent Taxonomic Revisions of Ostracoderms.

Sloan, Robert E. and Robert Jenness, University of Minnesota, Minneapolis and St. Paul. A Comparative Study of Milk Proteins and Sugars.

Sokoloff, Alexander, W. H. Miner Agricultural Research Institute, Chazy, New York. Parallelism in the Effect of a Gene in the Tenebrionids *Latheticus oryzae* Waterhouse and *Tribolium castaneum* Herbst.

Solbrig, Otto T., University of California, Berkeley. Speciation Evolution in the Genus *Gutierrezia* (Compositae).

Spiroff, Boris E. N., Loyola University, Chicago, Illinois. Philosophy of Evolution.

Stebbins, G. L., S. K. Jain, G. S. Khush, and S. S. Shah, University of California, Davis. Evolutionary Trends in the Development of Stomatal Complexes in Monocotyledons.

Strickberger, Monroe W., Columbia University, New York, New York. Changes in *Drosophila pseudoobscura* Adaptive to Experimental Environments.

Whiting, P. W., University of Pennsylvania, Philadelphia. *Mormoniella* and the Evolution of the Gene.

Williams, George C., Michigan State University, East Lansing. Reproductive Compensation and the Frequencies of Lethal Genes.